BTAG Meeting Agenda

1216484 - R8 SDMS

December 8th, 2011
US Fish & Wildlife Service Offices
585 Shepard Way, Helena, MT.

Objectives: 1) Summarize data collected in 2011
2) Determine if additional fish toxicity tests are feasible, and if so how these tests will be generally designed
3) Determine if amphibian toxicity tests are feasible, and if so are revisions to the existing study design and protocol necessary
4) Determine if studies to evaluate exposures of avian receptors to LA is warranted, and if so how these studies will be designed

8:00-8:15AM Introduction

8:15-9:00AM

I. Avian Exposure
   a. Discuss available data/reports – *Dan Wall, Dr. Wideman, Anne Fairbrother*
   b. Determine if avian exposure studies are warranted - *BTAG discussion*

9:00-9:30AM

II. Water Quality in Rainy Creek Watershed
   a. Overview of data collected in 2011 – *Christina Progess*
   b. Recommendations for continued sampling and analysis – *BTAG discussion*

9:30-12:00PM

III. Habitat Assessment
   a. Description of field activities – *Joe Volosin*
      [15 minute break]
   b. Interpretation of data collected – *Don Wall*
   c. Recommended path forward – *BTAG discussion*

12:00-1:00PM Lunch

1:00-3:30PM

IV. Toxicity Testing
   a. Review issues from previous tests – *Bill Brattin*
   b. Discuss options for future tests – *Don Wall*
   c. Recommended path forward – *BTAG discussion*
      [15 minute break]

3:45-5:00PM Open discussion

5:00PM Adjourn

Sign In Sheet
Libby OU3 BTAG meeting
12/8/11 Helena, MT

Name	Email
Sherry Skipper	skipper.sherry@epa.gov
Richard Henry	richard_henry@fws.gov
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Bob Medler	robert.j.medler@grace.com
Bill BRATTIN	brattin@SRC INC.com
Sue Robinson	scrobinson@golder.com
Doug Fort	djfort@fortlabs.com
LYNN WOODBURY	woodburyl@cdm.com
Christina Progess	progess.christina@epa.gov

On the phone:

Bob Marriam
Bill Stubblefield
Allison Cardwell
Ann Fairbrother
Dr. Wideman
Dave Charters

BTAG meeting Notes 12/8/11

Avian discussion

- <2um particles may be retained in airway, particles >2um easily expelled
- particles trapped in epithelial cells in Bronchii
- thinks particles handled nicely by birds
- duff particles are of respirable size
- uncertain how much would be respired by ground dwelling birds
- Dr. Widema doesn't feel that there are any detrimental effects to birds
- fibers walled off by fibrous tissue, not so damaging to avian lung
- Mt. St. Helens bird experiment, San Diego Zoo experiment
  - showed granulomas, no evidence of ill effect
- birds can operate on only one lung
- Wild fowl more robust than chickens, Dr. Widema thinks granuloma wouldn't detrimentally affect wild birds
- biggest uncertainty how readily is it respirable in colloidal form that is respired into air sacks
- fibrosis in avian lung doesn't make it work any harder
- if protective of mammals, then protective of birds - says Dr. Widema
- Dr. Widema doesn't think ingestion will be problem because epithelial cells get sloughed off every 24-48 hrs

Surface Water

base flow Carney & Fleetwood ~ 40 GPM base flow
3 broad things to consider - is sampling needed for tox tests? HH MCL assessment? Options for engineering solutions?

Habitat data

no young of year in lower stretches of Rainy Creek
much lower concentrations of trout in Lower Rainy Creek
low % woody debris in LRC
% biomass & trout pop well correlated w/ spawning gravel amt
URC trout pop isolated by barriers but clearly reproducing
HSI doesn't reveal diff between reaches that explain pop diff.
additional habitat data not likely to help evaluate this issue

TOX test

Previous pilot study results: fibers lost after 8 hrs,
by 24 hrs loss = 40%, 48 hrs = 70%
we can create dilutions w/o measurable fiber loss
options:
- remove fish from tank - create way to not stress fish
  - allison tried & saw fish stress
- in-stream tox test } not control
- flow through test } concentration, no dose response

bill S. supports in-stream tox test
- multiple life stages - larval fish for several weeks

Dave R. - collect fish at diff life stages, do necropsy & histology to see if an affect - look in diff areas in streams given Joe V. Wonath
Bill S. forward info on study designs for diff life stages in in-situ test

3 part study {
30 day tox test w/ critical life stage in lab
in-situ test w/ cages w/ older fish
necropsies of fish in field

Doug Fort & Sue work up rough design for amphibian lab sediment test

amphibia
field collection - variety of stages
Sed only tox test - target highest conc sed

fish
caged fish studies
get info from Bill S. - egg exposure, which life stage most appropriate?
- conf. call in near future
necropsy of native fish - gills, lateral line, fillets? (talk to Dave & Deb) - Sue put together design for this - me send out to group
EPA get DQO's for tox studies
Permits for collection - Sue will work permits, need plan before apply for permit

SW samples to coincide w/ caged tox test locations (fish & amph)
collect both free & total CA
- Sample sediment for amphib locations
- Sample water in tanks during lab amphibia test (total & free

Follow up conversation w/ Bill S Regarding in-situ test design
Send

Libby OU3 BTAG Meeting Notes - December 8, 2011

Attendees:
Christina Progess, EPA RPM
Dan Wall, EPA
Sherry Skipper, EPA
Richard Henry, USFWS
Karen Nelson, USFWS
John Podolinsky, MDEQ
Carolyn Rutland, MDEQ
Bob Medler, W.R. Grace
Sue Robinson, Golder Associates
Joseph Volosin, Anchor QEA
Doug Fort, Fort Environmental
Bill Brattin, SRC
Lynn Woodbury, CDM

Via phone -
David Charters, EPA
Bob Marriam, Remedium
Robert Wideman, University of Arkansas
John Garr, MWH Americas
Anne Fairbrother, Exponent
Bill Stubblefield, Oregon State University
Allison Cardwell, Oregon State University

Avian Exposure

Dr. Wideman provided an overview of his expert opinion on the susceptibility of birds to asbestos inhalation (see file: *Avian Respiration Summary for EPA.pdf*).

- Unlike mammals, birds have non-inflating lungs, thus fibrosis is not a key response. Granuloma formation and a "walling off" response would be the expected consequence of asbestos exposure in birds.
- LA structures in duff are of a respirable size, but the majority would be expected to be cleared via the mucocillary escalator.
- Ingestion effects on the gastrointestinal tract are likely to be minimal due to the daily sloughing of epithelial cells.
- Wild birds are likely to be even more robust than broiler chickens (which are one of the most sensitive bird species).
- Mammals are likely to be more sensitive to particulate inhalation relative to birds.
- Protection of mammalian species will be protective of bird species.

BTAG agreed that no further investigation of bird exposures is deemed necessary at this time.

Water Quality

Christina Progess presented measured asbestos surface water concentrations and flow data collected in 2011 (see file: *OU3 IV-B SW Results_11-16-11.pdf*). Samples were collected from mid-April to September (18 rounds) at 4 stations (TP, CC-2, LRC-2, LRC-6). She also presented data from 2007 and 2008 for the purposes of comparison with the 2011 results (see file: *OU3 SW 2007-2008 vs 2011.pdf*).

- There is a temporal pattern, with concentrations generally tending to increase during high flow and decrease during low flow, with peak levels measured in the spring.
- Several high results likely illustrate the effect of suspended sediment in the water sample; the source of this sediment suspension is not known, but field crews have not

reported any deviations from protocol that would explain the high values. Natural causes (i.e., bioturbation, wind, etc.) are die suspected cause..

- Although samples for the analysis of "free fibers" were collected, nearly all samples have been archived for possible future analysis. Samples are archived on dry filters and are stable indefinitely.

BTAG agreed that analysis of "free" fibers in surface water is not warranted at this time; these samples should continue to be held in archive for possible future analysis.

- Several samples exceed the drinking water MCL (7 MFL for fibers longer than 10 um). URC and Kootenai River are designated as drinking water sources (A1), while LRC is not designated as drinking water source (C1). MDEQ attorney is currently evaluating the applicability of the MCL as an ARAR to each of these reaches.
- Available surface water data from the Kootenai River were collected under low flow conditions in 2008. Previously collected surface water samples were not ozone/UV treated prior to analysis.
- Future sampling locations (near bank, within channel) and number of samples collected will depend upon MCL point-of-compliance and statistic of compliance (annual average). *John Podolinsky will follow-up with MDEQ water compliance folks to determine the appropriate application of the MCL (i.e., point of compliance, statistic of compliance).*

BTAG agreed that additional sampling to characterize asbestos surface water concentrations in the Kootenai River under high/low flow conditions is needed. CDM/SRC will develop a draft SAP for this sampling program.

- MWH is currently evaluating strategies for re-routing Rainy Creek around the tailings dam and removing the Mill Pond. Spillways near dam (and possible toe drains) may need some work.

BTAG agreed that additional sampling to establish a baseline condition, prior to any stream re-routing efforts, is not necessary.

<u>Aquatic Habitat Assessment</u>
Joe Volosin presented the results of the stream pool classification and pool temperature monitoring effort conducted in 2011.

- URC tended to be cooler than LRC. Noisy Creek tended to be cooler than Bobtail Creek (LRC was similar to Bobtail). LRC temperature tended to decrease with increasing distance downstream (possibly due to groundwater influence).
- Temporal pattems in surface water temperatures tend to mimic air temperature pattems.
- URC-1A had fhe most pools. Only Noisy Creek had a Class 1 pool. Most sites dominated by Class 2 pools (especially LRC), which is not unexpected for streams of the size present at the site.

- LRC is effectively isolated from the Kootenai River due to a hanging culvert. Immigration of fish from the UCR is possible during high water conditions (water going over spillway at the tailings impoundment), but upward movement from LRC into URC is not possible.

Aquatic Habitat Modeling

Dan Wall presented the results of aquatic habitat modeling efforts using the Habitat Suitability Index (HSI) model for rainbow trout (see files: *Habitat data_Wall.pdf* and *HSI.pdf*). He also presented a series of figures that compared fish population density and biomass to various habitat metrics.

- Fish population density is higher in URC compared to LRC; young-of-the-year (YOY) appear to be low or absent in LRC.
- Previous BTAG meeting identified two important habitat variables for which data were not available: 1) % pools, 2) pool depth/temperature. Obtaining these habitat variables was the purpose of the 2011 sampling effort.
- HSI model has several lifestage components - egg, fry, juvenile, adult, and "other" (food base quality). Model output is a score (0-1); model defaults to a minimum value if certain variables are limiting (e.g., pool habitat is a limiting variable for the juvenile model).
- There appears to be little correlation of lifestage HSI scores to fish population estimates. Some individual habitat metrics appear to have fairly good correlations with population estimates (e.g., % spawning gravel, % woody debris). Other habitat variables do not.
- HSI is only a coarse tool with insufficient resolution to distinguish between OU3 sampling locations. Collection of additional habitat data will probably not explain fish population differences.

BTAG agreed that additional data collection of aquatic habitat information is not necessary at this time.

Fish Toxicity Testing

Bill Brattin provided an overview of the outcomes of the fish pilot study and the follow-on PCM pilot study (see file: *Tech Memo on Fish Pilot Study v5.doc*).

- LA concentrations in the spiked water used in the fish pilot study were about 500x too low (expected = 10 BFL, observed = 0.02 BFL). LA concentrations in the Stock A used to spike the water were about 50x too low (expected = 10,000 BFL, observed ~ 200 BFL). Temporal evaluation of concentration showed fiber loss begins at about 24 hours.
- The PCM pilot studies demonstrated that: 1) stock solutions are stable, 2) it is possible to perform dilutions without fiber loss, 3) transfer of solutions between beakers does not result in fiber loss, and 4) temporal evaluation of concentration showed fiber loss begins at about 8 hours.
- Consequently, while the PCM studies are encouraging, they do not provide an explanation for the low concentrations achieved in the stock vials or in the exposure chambers in the fish pilot study.

The BTAG discussed whether additional fish toxicity testing shoulld be attempted and, if so, what study design changes would be appropriate based on the results of previous pilot studies.

- Based on available surface water data for Rainy Creek, it may be appropriate to utilize lower LA concentrations in the spiking water. This may allow the use of existing LA stock solutions, but there is no evidence that the dilution issues would be resolved by the use of lower concentrations.
- Bob Medler noted that one goal is to meet the MCL in site creeks (7 MFL for fibers longer than 10 um); assuming about 10% of all structures this would be a total LA water concentration of about 70 MFL.
- Water change-out frequency would be needed on a daily basis. Simply adding new spiking water to the old aquarium would not be effective because of the organic sludge build-up on the aquarium walls. The group discussed water change-out options that would allow fish to be moved from the old aquarium to the new aquarium with minimal stress to the organisms (e.g., use of a aquarium "basket" that can be lifted out and inverted over the new aquarium).

BTAG agreed that additional laboratory-based fish toxicity tests would not be attempted at this time. Instead, other lines of evidence would be explored to provide information on fish population exposure and effect (see below).

<u>Other options to assess effects on fish:</u>

On-Site Toxicity Testing Laboratory – Using EPA's mobile laboratory at an on-site location, perform a static-renewal or flow-through fish toxicity study of exposures to creek water. The chief limitation of this type of study is that the exposure concentration cannot be controlled (i.e., the exposure is what the water concentration is). Additionally, exposure chambers and associates equipment that contacts water could result in reducing water column concentration similar to what was seen in laboratory tests.

BTAG agreed that an on-site static-renewal or flow-through study with the mobile lab will not be conducted at this time.

Caged Fish Study – Place fish at various lifestages in cages in various creek locations across the site and determine if there are differences in survival between on-site and off-site following long-term (weeks/months) exposure to site conditions. Dan Wall noted that MT Fish Wildlife and Parks has had some success using caged studies in Montana. Bill Stubblefield provided an overview of caged study designs developed by the State of Washington and British Columbia that allow for fhe evaluation of a broad range of fish lifestages (including eggs, sac fry). If early lifestages are utilized, no feeding is necessary (egg yolk will provide sufficient nourishment). If later lifestages are utilized and growth is an endpoint of interest, fish will need to be fed regularly. Caged fish study has limitations similar to those presented above for the on-site toxicity test. Significant limitations to this approach were identified, including: 1) the inability to control exposure concentrations or environmental variables, 2) caging is potentially stressful to the fish, and 3) requires significant maintenance.

Bill Stubblefield will forward the caged fish study methods to Christina Progess for distribution to the BTAG for consideration in the design of a caged fish study at OU3.

BTAG agreed that, despite limitations, conducting a caged fish study has the highest likelihood of success and should be pursued but that additional discussion is necessary following the receipt of the caged study methodology to design the study. It is anticipated that, as part of this caged study, collocated surface water measurements - total LA and free (for archive) - would be needed.

In-Situ Effects Assessment - Collection of fish from on-site and off-site locations and determine if there are differences in the frequency or severity of observed effects (e.g., lesions, deformities, histological metrics). These effects data would add to the weight of evidence to determine if unexpected adverse effects occur in on-site fish and to distinguish the potential effects of LA vs differences in habitat. As part of this sampling effort, *Remedium agreed to collect larger fish (edible) for EPA to analyze for LA concentration.*

Christina Progess will provide input on potential data needs to support any human health evaluation.

BTAG agreed that an in-situ effects assessment would provide another line of evidence that could be used to support the weight of evidence for the risk assessment. Sue Robinson/Joe Volosin will prepare a brief (2-3 page) initial proposed study design for review by the BTAG.

Golder Associates will be responsible for applying for the appropriate fish collection permits with Montana Fish, Wildlife, and Parks.

Amphibian Toxicity Testing

The group discussed the feasibility of completing the amphibian toxicity tests, in light of the challenges faced in the fish toxicity tests.

- Doug Fort provided input to the BTAG on the relative contribution of each exposure medium (surface water, sediment) to amphibian exposures. He noted that, while the surface water toxicity test would be subject to all the issues identified during the fish toxicity tests, that there was no reason that the sediment only toxicity tests could not be performed.
- Doug proposed the following for the sediment-only toxicity test:
    - o Use of *Rana* spp. (not bullfrog)
    - o Sediment to overlying water ratio of 1:4, with an overlying water depth of about 6 inches
    - o No overlying water change-out
- Limitation in this type of study is that it would not be possible to establish a dose-response relationship based on the results for a single sediment collection location (e.g., TP-TOE2).

BTAG agreed that a spiked sediment toxicity test will not be performed due to concerns about representativeness of exposure and the high mass requirements that would be needed.

BTAG agreed that an amphibian toxicity test will be performed using site sediment collected from an area with high LA concentrations (by PLM-VE). Water used to overlay the sediment will be lab water (no added LA). Doug Fort will revise the existing amphibian toxicity test study design to incorporate changes discussed at the meeting and submit for review by the BTAG. It is anticipated that samples of overlying water will be collected occasionally for analysis of total LA and that samples for analysis of "free" LA fibers would be collected but archived for potential future analysis.

<u>Other options to assess effects on amphibians:</u>

In-Situ Effects Assessment - Collection of amphibians of varying lifestages from on-site and off-site locations and determine if there are differences in the frequency or severity of observed effects (e.g., lesions, defonnities). Karen Nelson noted that one potential off-site reference area is a refuge located about 1 hour away from Libby. In addition, data in the lIterature may also provide useful information on baseline conditions. These effects data would add to the weight of evidence to determine if unexpected adverse effects occur in on-site amphibians.

BTAG agreed that an in-situ effects assessment would provide another line of evidence that could be used to support the weight of evidence for the amphibian risk assessment Doug Fort will prepare an initial proposed study design for review by the BTAG. It is anticipated that, as part of this in-situ assessment, collocated surfoce water measurements - total LA and free (for archive) - and sediment measurements would be needed.

Golder Associates will he responsible for applying for the appropriate fish collection permits with Montana Fish, Wildlifo, and Parks.

<u>Miscellaneous</u>

- The collection of overbank sediment data from the Kootenai River downstream of Rainy Creek will be addressed as part of the nature & extent characterization efforts.
- *BTAG agreed that, while the off-site reference streams (Noisy Creek and Bobtail Creek) are somewhat different in their habitat characteristics from the on-site creeks, they bracket expected habitat conditions at the site and will be retained for the purposes of future studies.*
- Autosampler surface water samples collected in 2008 are being held in archive at EMSL in Libby. It is unclear if these samples would be able to be analyzed for asbestos or if the results wlll be useful for the purposes of supporting the ecological risk assessment. *Bill Brattin will discuss the foasibility of analysis with Ron Mahoney (EMSL, Libby).*

Libby OU3 Rainbow Trout Habitat Suitability Index (HSI)

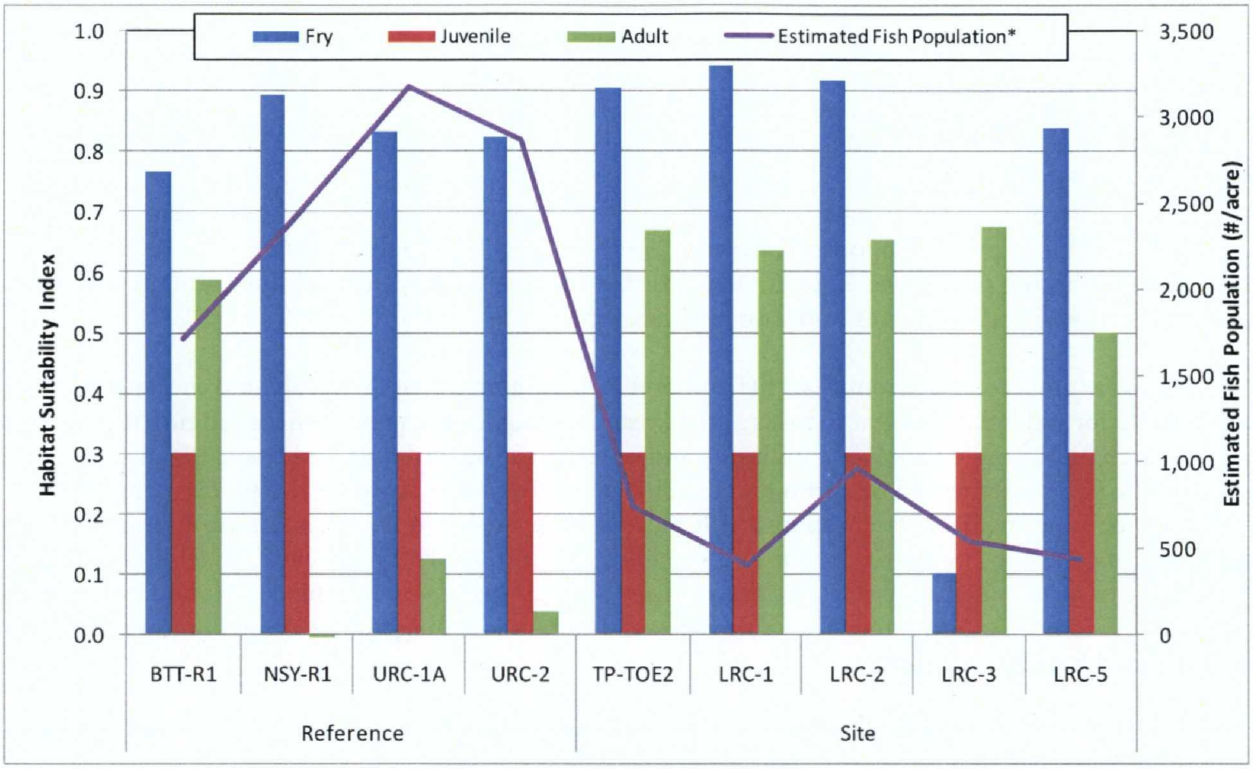
The Habitat Suitability Index (HSI) model for rainbow trout (Raleigh et al. 1984) was created to aid in identifying important habitat variables by utilizing species-habitat relationships. The species-habitat relationships were developed based on facts, ideas, and concepts obtained from research literature and expert reviews. Because the DOS program that was created to support the HSI calculations was not able to be used, the figures in the supporting documentation (Raleigh et al. 1984) were re-created in Excel. Formulas representing the species-habitat relationships were derived by fitting a line through data points that were selected from the figures in the documentation.

Four life stages (embryo, fry, juvenile, adult) and one "other"[1] component are evaluated in the model by utilizing data for individual habitat metrics and translating those values into indices ranging from zero to one, with zero indicating unsuitable conditions and one indicating optimal conditions. Ideally, the life stage-specific HSI scores would be combined to achieve one total HSI score for the species. For Libby OU3, data were only available to compute a HSI score for the fry, juvenile, and adult life stages. Data were insufficient to compute HSI scores for the embryo life stage and "other" component. In addition, data were either not available or only available for a subset of sampling locations. Hence, a total HSI score for the species could not be computed. The table below contains a summary of the HSI habitat metrics that were evaluated for each life stage.

Life Stage	Habitat Variable
Adult	Average water depth
	Percent in-stream cover
	Percent pools
	Pool class rating
Juvenile	Percent in-stream cover
	Percent pools
	Pool class rating
Fry	Percent substrate size class
	Percent pools
	Percent fines (<3 mm) in spawning areas

The HSI scores for the individual life stages are presented in the figure below. For the juvenile life stage, the HSI score for all stations is 0.3. This is because, if the minimum score for any metric is less than or equal to 0.3, then the minimum score is the juvenile life stage HSI score. In this case, the pool class rating becomes the driver for the juvenile life stage HSI score because it yields a score of 0.3 for all stations.

[1] The "other" component contains model variables for two subcomponents, water quality and food supply, that affect all life stages.



Reference:

Raleigh, R. F., T. Hickman, R. C. Solomon, and P. C. Nelson. 1984. Habitat Suitability Information: Rainbow Trout. U.S. Fish Wildl. SErv. FWS/OBS-82/10.60. 64 pp.

LIBBY OU3: PHASE IV PART B SURFACE WATER SAMPLING RESULTS (as of November 16, 2011)

Sampling Round		Sample Date	TP							CC-2							LRC-2							LRC-6						
			Index ID - Total	Total LA (MFL) ALL	LA > 10um (MFL)	Index ID - Free	Free LA (MFL)	Clumps (#/L)	Syringe Volume (mL)	Index ID - Total	Total LA (MFL) ALL	LA > 10um (MFL)	Index ID - Free	Free LA (MFL)	Clumps (#/L)	Syringe Volume (mL)	Index ID - Total	Total LA (MFL) ALL	LA > 10um (MFL)	Index ID - Free	Free LA (MFL)	Clumps (#/L)	Syringe Volume (mL)	Index ID - Total	Total LA (MFL) ALL	LA > 10um (MFL)	Index ID - Free	Free LA (MFL)	Clumps (#/L)	Syringe Volume (mL)
Weekly Sampling	Round 1	4/19/11	P4-50012	26	3.5	P4-50010	4.3	2.4E+05	50[a]	P4-50006	20	1.0	???			???	P4-50009	20	2.6	P4-50008	9.1	9.1E+04	10	P4-50003	68	13	???			???
	Round 2	4/26/11	P4-50028	27	3.0	P4-50029	16	6.1E+05	50	P4-50025	80	7.2	P4-50027	84	0	10	P4-50022	34	3.0	P4-50024	35.6	7.0E+05	10	P4-50013	138	27	P4-50017	45.0	1.3E+06	10
	Round 3	5/3/11	P4-50034	18	2.9	P4-50035	5	9.6E+04	50	P4-50037	36	3.3	P4-50039			10	P4-50031	92	20	P4-50033	41	1.2E+06	10	P4-50040	20	1.8	P4-50041	14	1.3E+05	50
	Round 4	5/10/11	P4-50058	154	28	P4-50059			50	P4-50055	80	6.4	P4-50056			50	P4-50052	51	9.0	P4-50053			50	P4-50043	119	27	P4-50044			50
	Round 5	5/17/11	P4-50070	25	1.7	P4-50071			50	P4-50067	249	7.5	P4-50068			50	P4-50064	66	5.3	P4-50065			50	P4-50061	276	55	P4-50062			50
	Round 6	5/24/11	P4-50088	20	1.8	P4-50089			50	P4-50085	51	2.5	P4-50086			50	P4-50082	41	3.2	P4-50083	51	0	50	P4-50079	130	15	P4-50080			50
	Round 7	5/31/11	P4-50106	72	16	P4-50107			50	P4-50097	57	4.6	P4-50098			50	P4-50094	37	1.1	P4-50095			50	P4-50091	24	1.8	P4-50092			50
	Round 8	6/7/11	P4-50118	14	2.1	P4-50119			50	P4-50115	24	1.3	P4-50116			50	P4-50112	19	2.5	P4-50113			50	P4-50109	26	2.0	P4-50110			50
	Round 9	6/14/11	P4-50136	126	10	P4-50137			50	P4-50133	25	2.3	P4-50134			50	P4-50124	6	0.29	P4-50125			50	P4-50121	40	5.6	P4-50122			50
	Round 10	6/28/11	P4-50148	31	5.7	P4-50149			50	P4-50145	15	1.5	P4-50146			50	P4-50142	15	2.5	P4-50143			50	P4-50139	29	2.8	P4-50140			50
	Round 11	7/5/11	P4-50166	29	2.8	P4-50167			50	P4-50163	13	1.5	P4-50164			50	P4-50160	13	2.4	P4-50161			50	P4-50151	44	6.2	P4-50152			50
	Round 12	7/12/11	P4-50178	33	3.3	P4-50179			50	P4-50175	12	0.87	P4-50176			50	P4-50172	10	1.1	P4-50173			50	P4-50169	20	3.0	P4-50170			50
	Round 13	7/19/11	P4-50196	0.8	0	P4-50197			50	P4-50193	11	1.5	P4-50194			50	P4-50190	18	2.2	P4-50191			50	P4-50181	0	0	P4-50182			50
	Round 14	7/26/11	P4-50208	11	0.55	P4-50209			50	P4-50205	38	6.8	P4-50206			50	P4-50202	27	3.2	P4-50203			50	P4-50199	20	1.2	P4-50200			50
Bi-weekly Sampling	Round 15	8/9/11	P4-50226	30	3.1	P4-50228			10	P4-50223	0.76	0.12	P4-50224			50	P4-50220	44	7.5	P4-50221			50	P4-50217	41	4.4	P4-50218			50
	Round 16	8/23/11	P4-50244	57	2.3	P4-50245			50	P4-50235	50	2.0	P4-50236			50	P4-50232	41	5.6	P4-50233			50	P4-50229	34	2.0	P4-50231			10
	Round 17	9/6/11	P4-50256	23	2.4	P4-50257			50	P4-50253	27	5.5	P4-50254			50	P4-50250	2.7	0.24	P4-50251			50	P4-50247	20	2.6	P4-50248			50
	Round 18	9/20/11	P4-50274	209	16	P4-50275			50	P4-50271	273	26	P4-50272			50	P4-50265	9.3	0.65	P4-50266			50	P4-50259	7.0	0.93	P4-50260			50
Opportunistic		11/9/11								P4-50272[b]	0.56	0																		

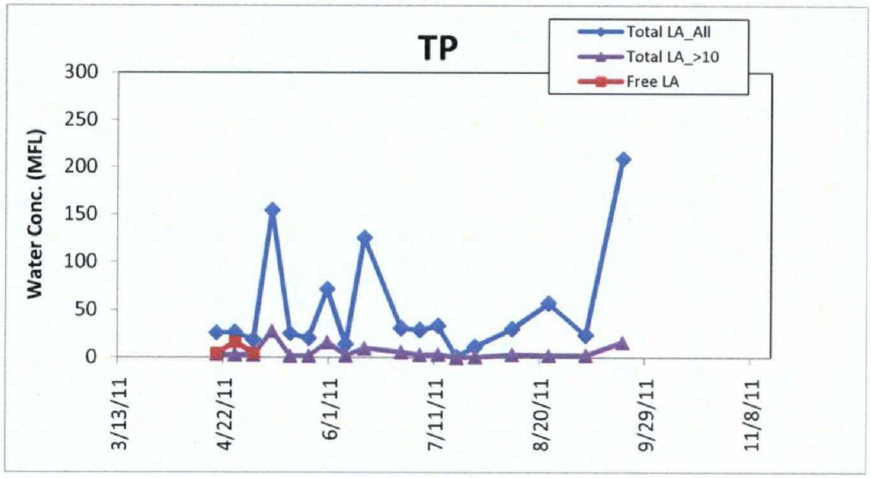
Footnotes:

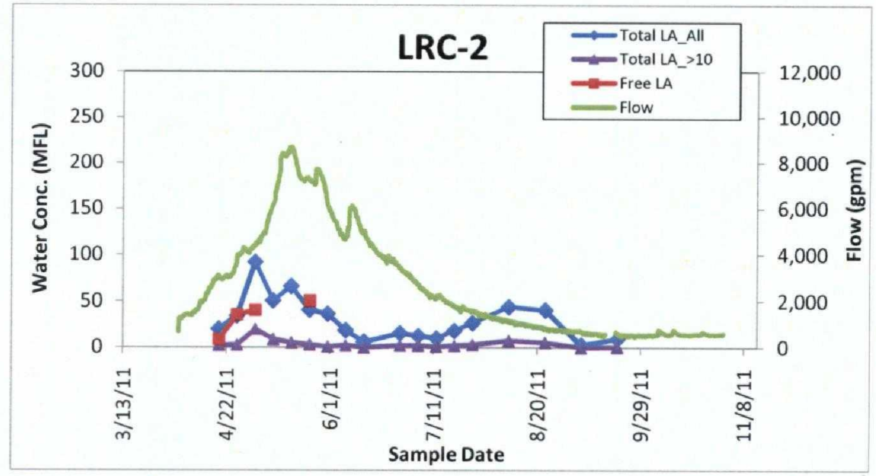
[a] 10 mL also analyzed (P4-50011); free LA = 19.6 MFL, clumps = 3.8E+05 #/L

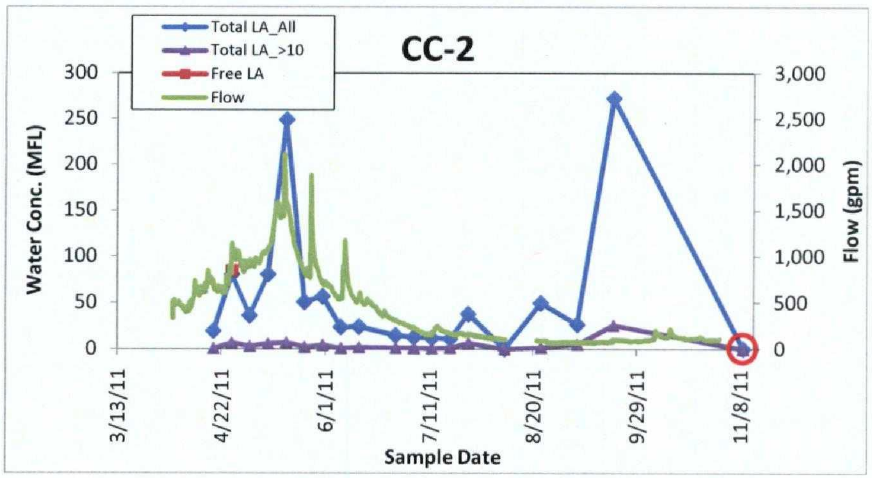
[b] Opportuinistic sample collected in response to elevated levels observed in Round 18.

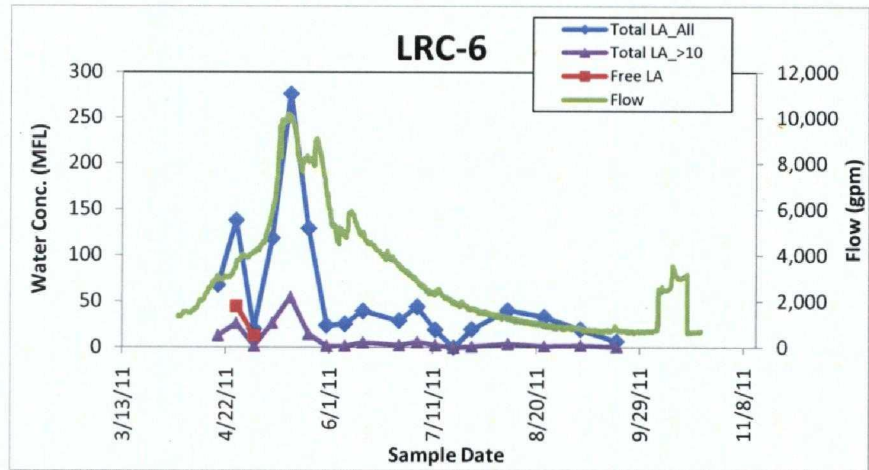
DRAFT - UNVALIDATED

LIBBY OU3: PHASE IV PART B SURFACE WATER SAMPLING RESULTS (as of November 16, 2011)







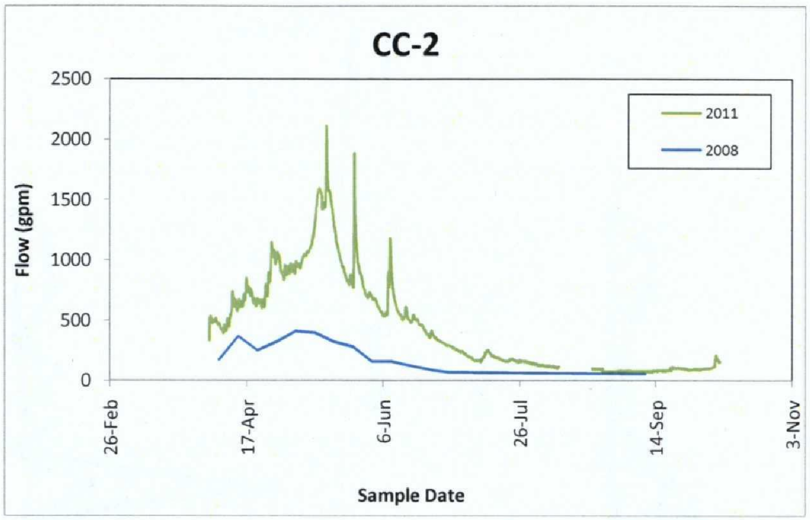


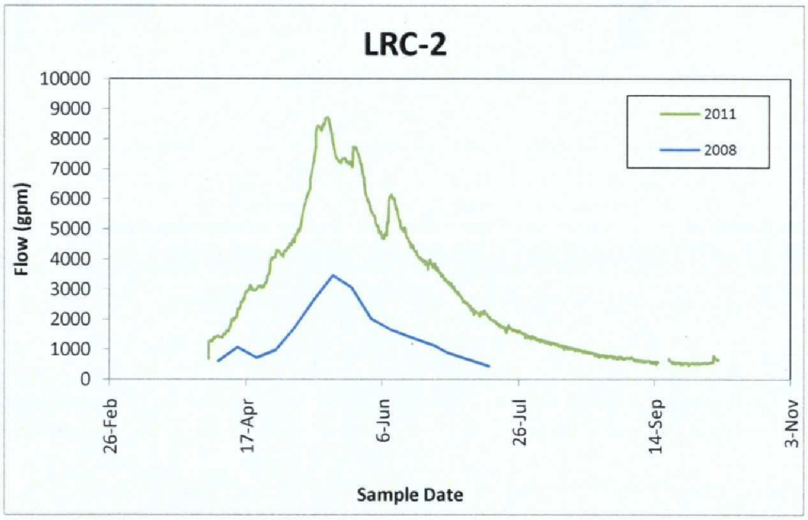
○ =opportunistic sample collected on 11/9/11.

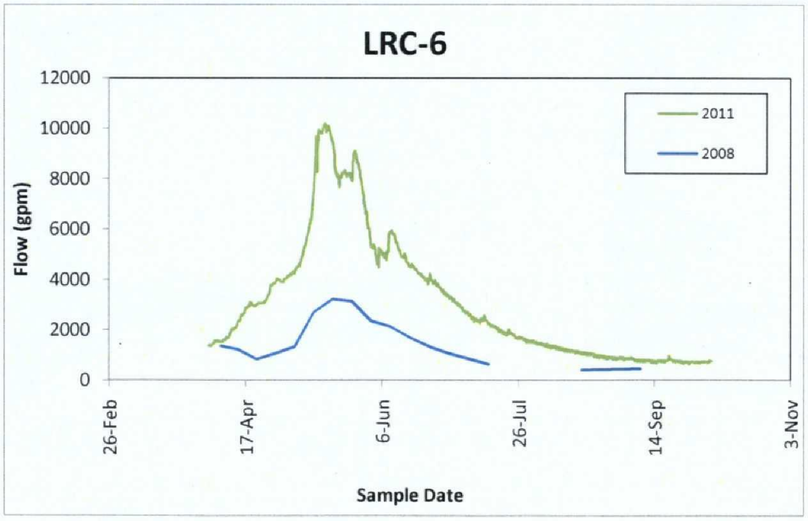
DRAFT - UNVALIDATED

COMPARISON OF 2008 SURFACE WATER RESULTS TO 2011 RESULTS

Flow (gpm)

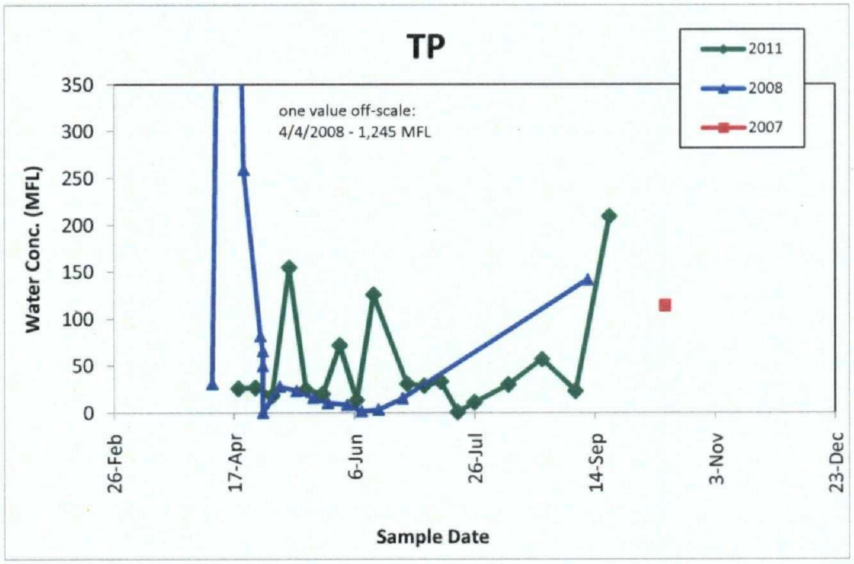


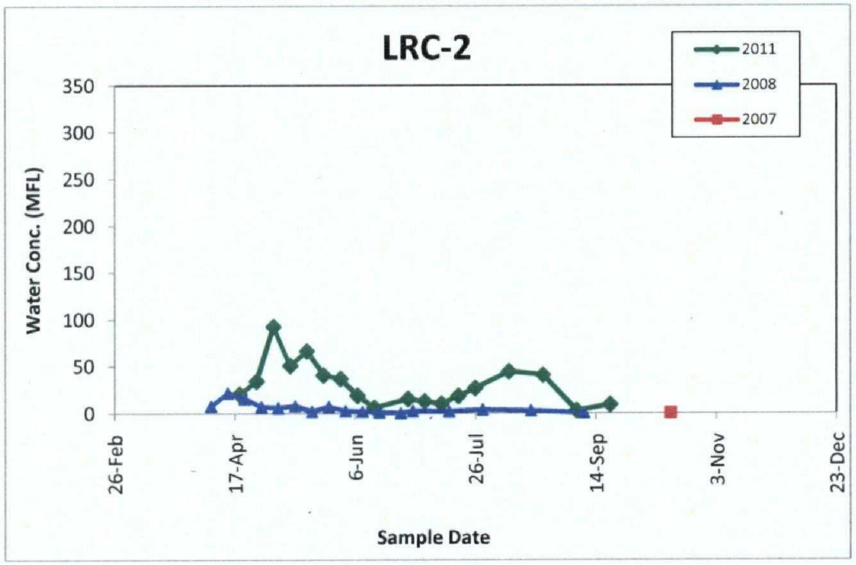


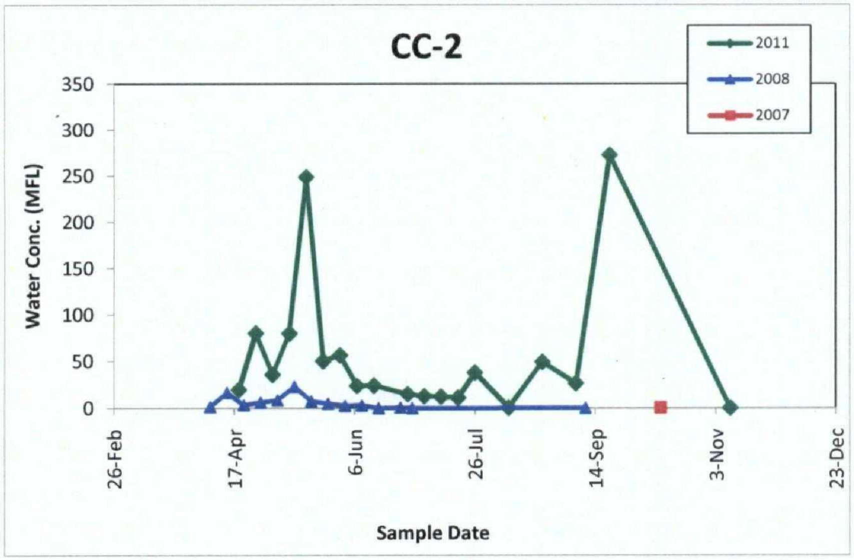


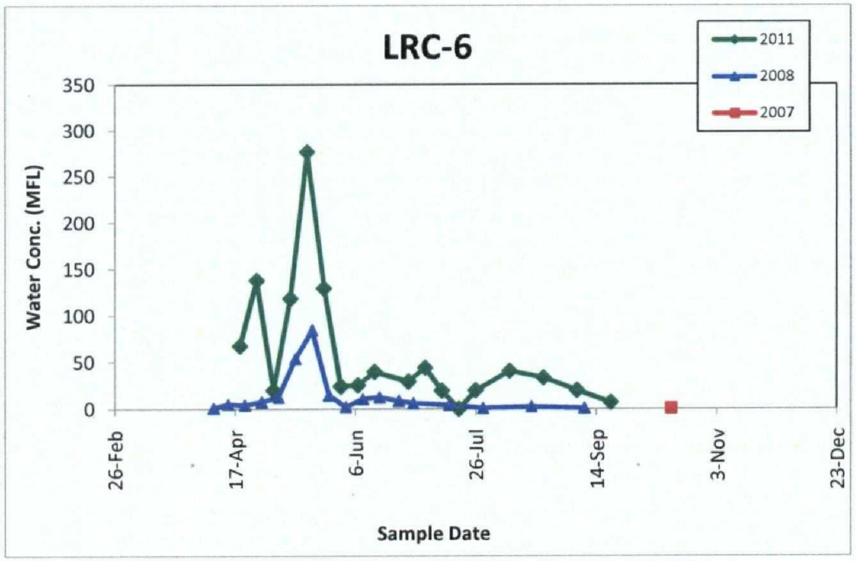
COMPARISON OF 2007-2008 SURFACE WATER RESULTS TO 2011 RESULTS

Total LA Water Concentration (MFL)









TECHNICAL MEMO

SUMMARY AND EVALUATION OF DATA FROM THE LIBBY OU3 FISH PILOT STUDY

L0 STUDY DESIGN

This study was designed to investigate the concentration of total and free Libby amphibole (LA) as a function of time under exposure conditions that will be used in the definitive fish toxicity test. The fish pilot study design is summarized in Figure L

Target exposure concentrations were 10, 1, 0.1 and 0.01 billion total LA fibers per liter (BFL), plus a zero control.

2.0 NAMING CONVENTIONS

Data reported by the laboratories used the following naming system:

Concentration:

Nominal Cone. (BFL)	"Dilution"
10	5
1	4
0.1	3
0.01	2
0	1

Replicate Chambers (Tanks): A, B, C

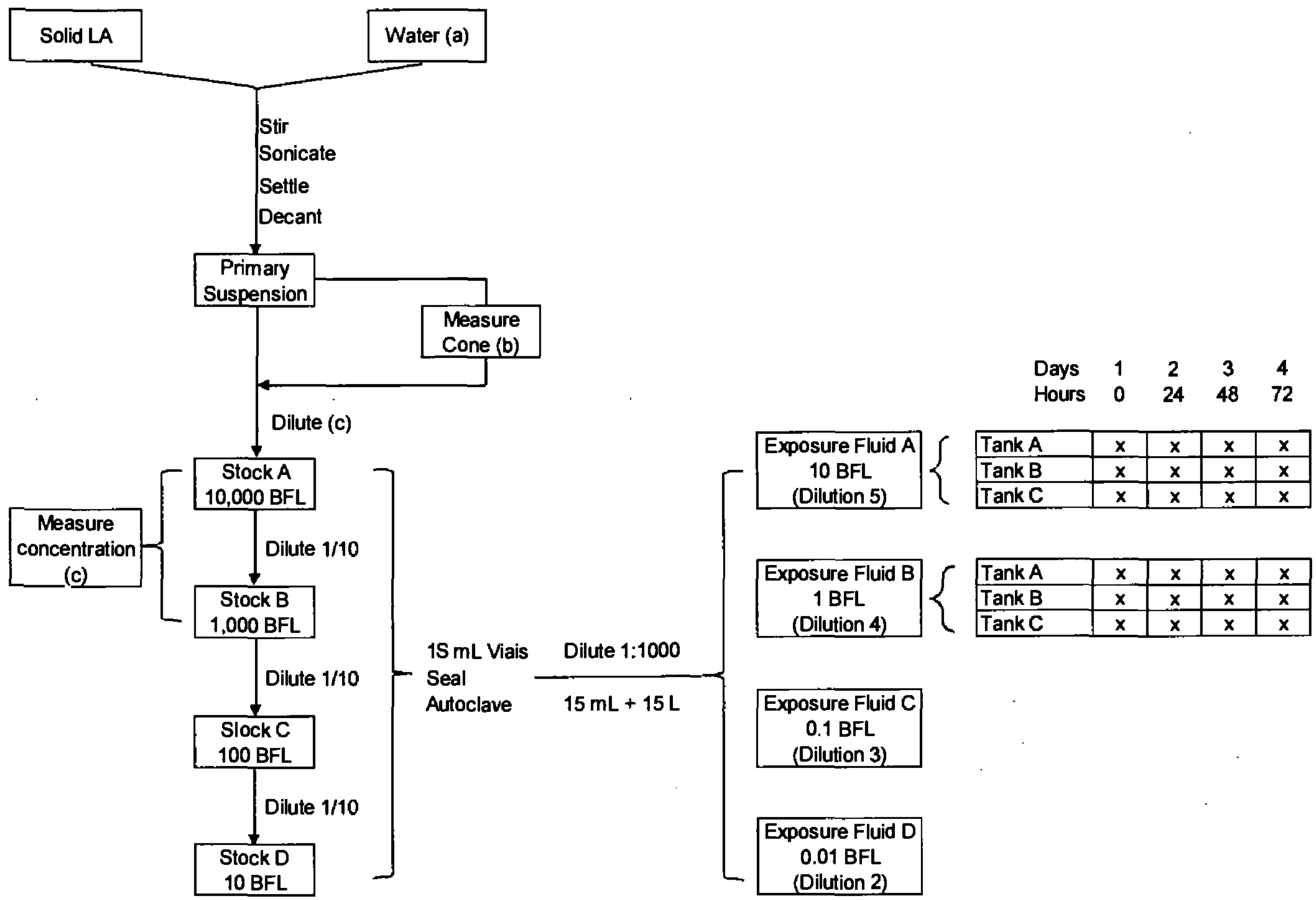
Days: 1, 2, 3, 4
Note: Day 1 = time zero (0 hours after water was placed in tank)
Day 2 = 24 hours after water was placed in tank, etc.

Analysis Type:
T = Total
F = Free

Example Sample Label:
4A2T01 = Dilution 4, Replicate Chamber A, Day 2, Total, Analysis 01

FIGURE 1.

FISH PILOT STUDY DESIGN



NOTES:

(a) Moderately hard reconsitituted lab water, ozonated before use
(b) See caiculation sheet 1
(c) See calculation sheet 2

3.0 OBSERVATIONS

Initial analysis of total LA from one of the high concentration tanks (i.e., Dilution 5; nominal concentration = 10 BFL) on day 1 (0 hours) revealed the actual concentration was much lower than expected:

Sample 5A1T01
LA Fibers Counted = 1
Grid Openings (GOs) Counted[1] = 15
Dilution Factor = 100
Total LA Concentration ≈ 0.02 BFL

In order to identify the reason for the unexpectedly low concentration, the concentration of the fluid in Stock A (the fluid used to spike the 10 BFL water) was measured. Stock B (the fluid used to spike the 1 BFL water) was also evaluated. The results (shown in Calculation Sheet 2) indicate that both Stock A and Stock B are substantially lower than expected:

Stock	Total LA Concentration (BFL)	
	Expected	Observed
Stock A	10,000	140-270*
Stock B	1,000	12

*Reported range across two different analytical laboratories

For Stock A, the discrepancy is about a factor of 50-fold (~200 BFL vs. 10,000 BFL). Because Stock B is prepared from Stock A by performing a 1/10 dilution, it would be expected that Stock B would also low by about the same factor. Using the data for Stock A from the same analytical laboratory that analyzed Stock B, the ratio is approximately 1:10 (12 BFL vs. 140 BFL), as expected. This suggests that the dilution of Stock A to produce Stock B did not yield unexpected results.

In order to detennine if an identifiable error occurred during the preparation and analysis of the primary suspension and the subsequent dilution to form Stock A, the data and the calculations from the laboratory were reviewed. No errors were identified (see Calculation Sheet 1).

[1] The initial analysis was based on an examination of 15 GOs; the result for the subsequent analysis (based on 50 GOs) is shown in Table 1.

CALCULATION SHEET 1		
Analysis of Primary Suspension		
N	79	
EFA	1282	mm2
GO	30	
Ago	0.0064	mm2
V	0.01	L
Dilution factor	1,000,000	
Cone	5.27E+13	f/L
	52748958	MFL
	52,749	BFL
Dilution of Primary Suspension		
V (primary susp.)	189.6	mL
V (final)	1000	mL
Cone (Stock A)	10,001	BFL

CALCULATION SHEET 2

STOCK A (Nominal = 10,000 BFL)

Parameter	Units	EMSL	Hygeia
N	f	153	233
EFA	mm2	360	346
GO	--	3	3
Ago	mm2	0.013	0.0099
V	L	0.01	0.01
Dil fact	--	1000	1000
Actual Conc	f/L	1.412E+11	2.714E+11
	MFL	1.41E+05	2.71E+05
	BFL	141	271

STOCK B (Nominal = 1,000 BFL)

Parameter	Units	EMSL	
N	f	132	
EFA	mm2	360	
GO	--	3	
Ago	mm2	0.013	
V	L	0.01	
Dil fact	--	100	
Actual Cone	f/L	1.218E+10	
	MFL	1.22E+04	
	BFL	12	

Table 1 summarizes the final data for all of the water samples from the fish pilot study analyzed for total LA from each of the two highest concentration levels (Dilution 5 and Dilution 4). Results for free LA are also shown for Dilution 5. Because of the unexpected findings, analysis of other samples for free LA was put on hold.

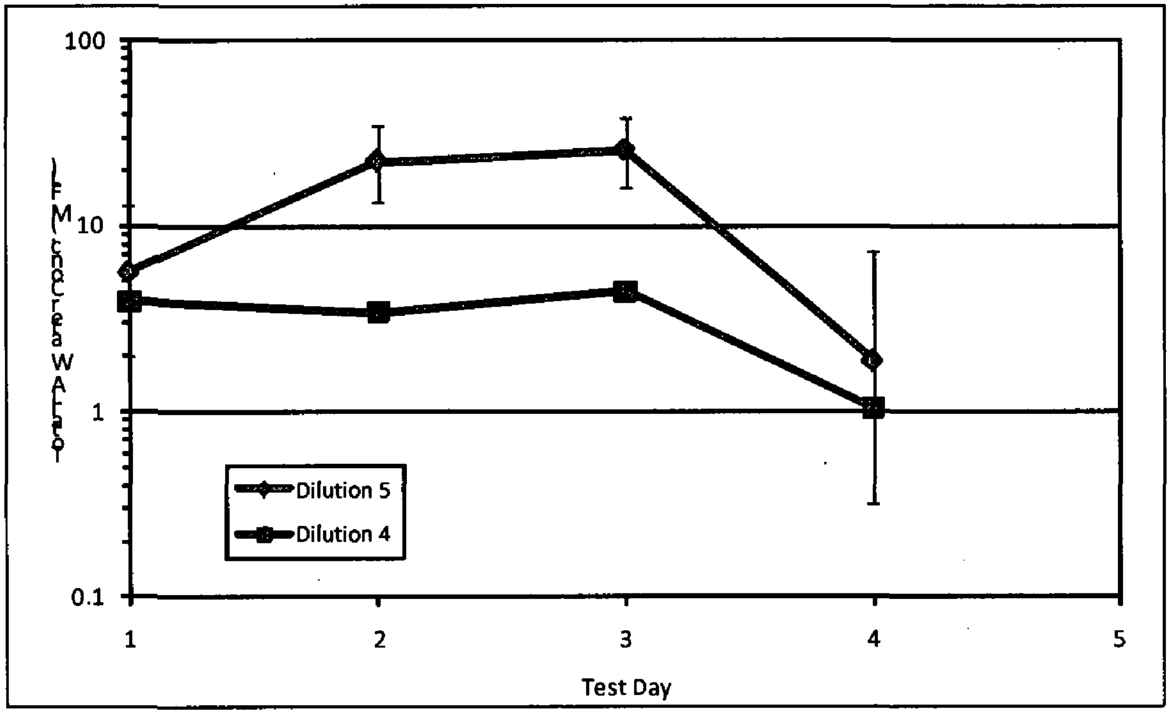
As shown in **Table 1**, there was substantial variability between replicates. Concentration estimates for free LA appear to be higher than for total LA, but because so few fibers were counted, the uncertainty bounds are very wide and the apparent difference is not likely to be real.

The results for total LA are plotted graphically in **Figure 2**. As seen, based on the mean across replicates, Dilution 4 tended to remain about constant for the first three measurements, but then decreased on day 4. Dilution 5 appeared to show a similar pattem, except for an unexpectedly low value on day 1. Because fewer fibers were counted during the analysis of Dilution 5 samples, the results for Dilution 5 are substantially less precise than for Dilution 4 (as illustrated by the wider confidence intervals on these concentrations).

TABLE 1. SUMMARY OF FISH PILOT STUDY DATA

			Replicate A						Replicate B						Replicate C						Pooled		
Analyte	Dilution	Day	N	GOs	V (mL)	DF	v (mL)	C (MFL)	N	GOs	V (mL)	DF	v (mL)	C (MFL)	N	GOs	V (mL)	DF	v (mL)	C (MFL)	N	v (mL)	C (MFL)
Total LA	5	1	1	50	10	100	1.8E-04	5.5	1	50	10	100	1.8E-04	5.5	1	50	10	100	1.8E-04	5.5	3	5.4E-04	5.5
		2	0	50	10	100	1.8E-04	0.0	9	50	10	100	1.8E-04	49.8	3	50	10	100	1.8E-04	16.6	12	5.4E-04	22.2
		3	3	50	10	100	1.8E-04	16.6	2	50	10	100	1.8E-04	11.1	9	52	10	100	1.9E-04	47.9	14	5.5E-04	25.5
		4	0	50	10	100	1.8E-04	0.0	0	50	10	100	1.8E-04	0.0	1	50	10	100	1.8E-04	5.5	1	5.4E-04	1.8
	4	1	100	35	10	1	1.3E-02	7.9	100	34	20	1	2.5E-02	4.1	87	50	20	1	3.6E-02	2.4	287	7.3E-02	3.9
		2	36	50	20	1	3.8E-02	1.0	101	25	20	1	1.8E-02	5.6	101	21	20	1	1.5E-02	6.7	238	6.9E-02	3.4
		3	103	28	20	1	2.0E-02	5.1	101	33	20	1	2.4E-02	4.2	103	35	20	1	2.5E-02	4.1	307	6.9E-02	4.4
		4	100	47	50	1	8.5E-02	1.2	32	50	50	1	9.0E-02	0.4	100	27	50	1	4.9E-02	2.1	232	2.2E-01	1.0
Free LA	5	1	1	4	10	100	1.0E-05	96.0	0	4	10	100	1.0E-05	0.0	0	4	10	100	1.0E-05	0.0	1	3.1E-05	32.0
		2	1	4	10	100	1.0E-05	96.0	1	4	10	100	1.0E-05	96.0	3	4	10	100	1.0E-05	287.9	5	3.1E-05	159.9
		3	1	4	10	100	1.0E-05	96.0	1	4	10	100	1.0E-05	96.0	3	4	10	100	1.0E-05	287.9	5	3.1E-05	159.9
		4	0	4	10	100	1.0E-05	0.0	0	4	10	100	1.0E-05	0.0	0	4	10	100	1.0E-05	0.0	0	3.1E-05	0.0
	4	1																					
		2																					
		3																					
		4																					

FIGURE 2. FISH PILOT STUDY RESULTS (TOTAL LA)



Estimating the observed concentration in Dilution 5 as about 20 million fibers per liter (MFL) and the observed concentration in Dilution 4 as about 4 MFL (based on results from days 1 to 3), it appears that both concentrations are lower than expected based on a 1:1,000 dilution of Stock A and Stock B:

Dilution	Concentration (MFL)	
	Expected	Observed
Dilution 5	~200*	~20
Dilution 4	12**	~4

*Based on a 1:1,000 dilution of Stock A (200 BFL)
**Based on a 1:1,000 dilution of Stock B (12 BFL)

The Dilution 5 concentration is about 10-fold lower than expected and the Dilution 4 concentration is about 3-fold lower than expected.

Discussion

These data indicate that an apparent loss of fibers occurred at each of two steps in the performance of the fish pilot study:

- The first apparent loss occurred somewhere between the preparation of the primary stock suspension and the creation of the sealed vials of Stock A.
- The second apparent loss occurred somewhere during the process of diluting the stock solutions and placing the water into the exposure chambers (tanks).

Taken at face value, the data suggest that losses occur during dilution steps. However, it appears that Stock A was able to be diluted to Stock B without substantial loss. Consequently, no likely hypothesis for this unexpected behavior has been identified.

- All calculations have been checked (several times, by different people), and it does not seem reasonable to suspect the results are due to math errors.
- Because these apparent losses were present at time zero (day 1), it seems unlikely that the loss is due to clumping or binding of fibers to vessel walls (at least binding due to organic growth). This is supported by the view that decreases in concentration from time zero did not become apparent until day 4.
- Binding of fibers to vessel walls due to other forces (besides organic growth) might be suspected, but it seems very unlikely that 95% of the material in a Stock vial could bind to the walls without being observed. In addition, such behavior would be totally unexpected in the presence of an aqueous salt solution.

FOLLOW-ON PCM PILOT STUDY

In order to determine if it is possible to perform even the most basic operations with LA suspensions, a follow-on pilot study was performed. The basic design was as follows:

1) One vial of Stock B was vigorously mixed by hand-shaking for 1 minute.

2) From this vial, a sample of about 3 milliliters (mL) was withdrawn and added to a 1-L Erlenmeyer flask containing about 900 mL of moderately hard reconstituted laboratory water (MHRLW). This dilution (expected to be about 36 MFL total LA) was well mixed with a magnetic stirrer. This was referred to as ***Fluid 1 (Flask #1).***

3) During mixing, 100 mL of this fluid were removed and diluted to 1,000 mL. The resulting dilution (expected to be about 3.6 MFL) was placed into a second 1-L Erlenmeyer flask and well mixed with a magnetic stir bar. This was referred to as ***Fluid 2 (Flask #1).***

4) At time = 0, 8, 24, and 48 hours, each fluid was well-mixed with a magnetic stirrer and three 10 mL aliquots were removed from each flask. These aliquots were filtered through 0.2 micrometer (µm) mixed cellulose ester (MCE) filters (25 millimeter [mm] diameter).

5) In addition, at time = 0 hours, a 50 mL aliquot was removed from the Fluid 1 flask and placed in a second flask. This flask was swirled by hand for several minutes, and then three 10 mL aliquots were removed from this second flask and filtered through 0.2 µm MCE filters (25 mm diameter). These filters were referred to as ***Fluid 1 (Flask #2)***.

6) All filters were analyzed by phase contrast microscopy (PCM) using NIOSH 7400, counting 100 fields of view (FOVs) or 100 structures, whichever came first. In addition, one filter from Fluid 1 (Flask #1) at time = 0 hours was also analyzed by TEM.

When this study was initially performed, some omissions from the study design occurred. Consequently, the study was run a second time.

Results are summarized below.

1. <u>Concentration of Fluid 1 Flask #1 (Time = 0 hours)</u>

	TEM (MFL)	PCM (MFL)
Run 1	46	14
Run 2	24	13
Expected	36 (a)	≈ 13 (b)

(a) Calculated from results from fish pilot study
(b) Assumes PCM LA ≈ 35% of total LA

Conclude: Dilutions prepared from Stock B are somewhat variable, but appear to be stable over time.

2. <u>Fluid 1 Flask #1 vs. Fluid 1 Flask #2 (Run 2, Time = 0 hours)</u>

Flask #1: 13.4 PCM MFL
Flask #2: 12.7 PCM MFL

Conclude: It is possible to perform a simple operation, such as pouring a suspension from one container to another, without fiber loss.

3. <u>Fluid 1 vs. Fluid 2 (Time = 0 hours)</u>

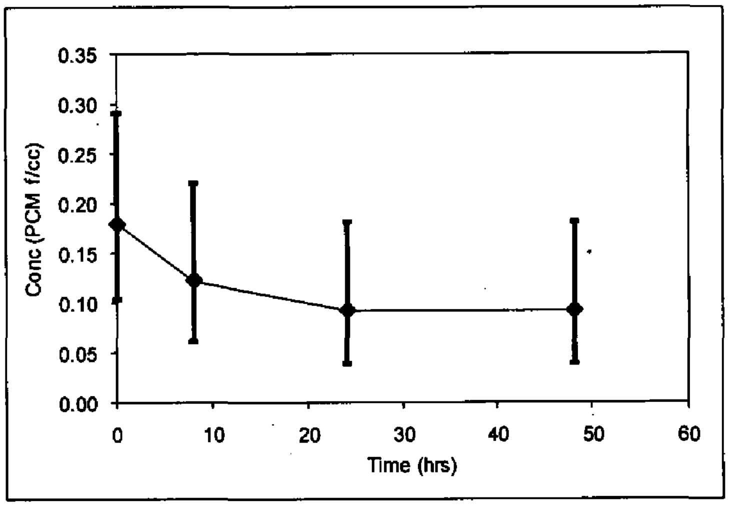
	Fluid 1 Observed (PCM MFL)	Fluid 2 (PCM MFL)	
		Observed	Expected
Run 1	14	0.18	1.4
Run 2	13	1.5	1.3

In Run 1, the concentration in Fluid 2 is lower than expected. The ratio (about 100:1) suggests a possible error in preparation of Fluid 2 (10 mL diluted to 1000 mL, rather than 100 mL diluted to 1000 mL). In Run 2, the concentration in Fluid 2 is 1/10 that of Fluid 1, as expected.

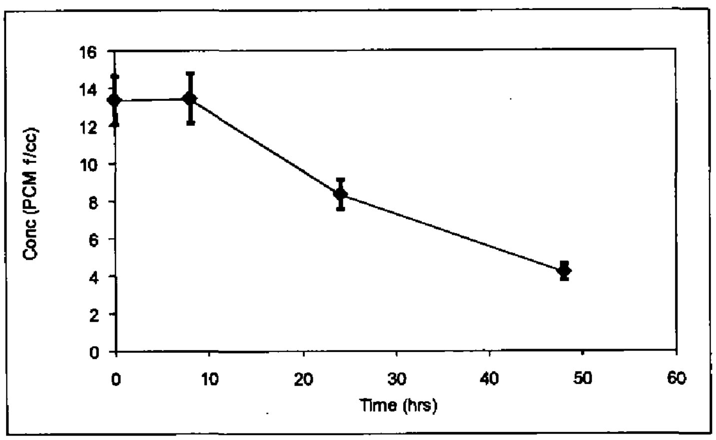
Conclude: Assuming the results from Run 1 are due to a dilution error, then it is concluded that dilutions can be prepared without fiber loss.

4. <u>Stability over Time</u>

Run 1, Fluid 2:



Run 2, Fluid 1:



Results from Run 1 (Fluid 2) indicate a drop in concentration over time, but there were too few coumts to be sure (confidence intervals are wide). Results from Run 2 (Fluid 1) were much clearer – there is a time-dependent decrease in fiber concentration beginning after about 8 hours.

Conclude: Even under "ideal" conditions (freshly ozonated water, no fish, no food), fibers begin to be lost after 8 hours. By 24 hours, the loss is about 40% and by 48 hours the loss is about 70%.

QUESTIONS FOR BTAG DISCUSSION

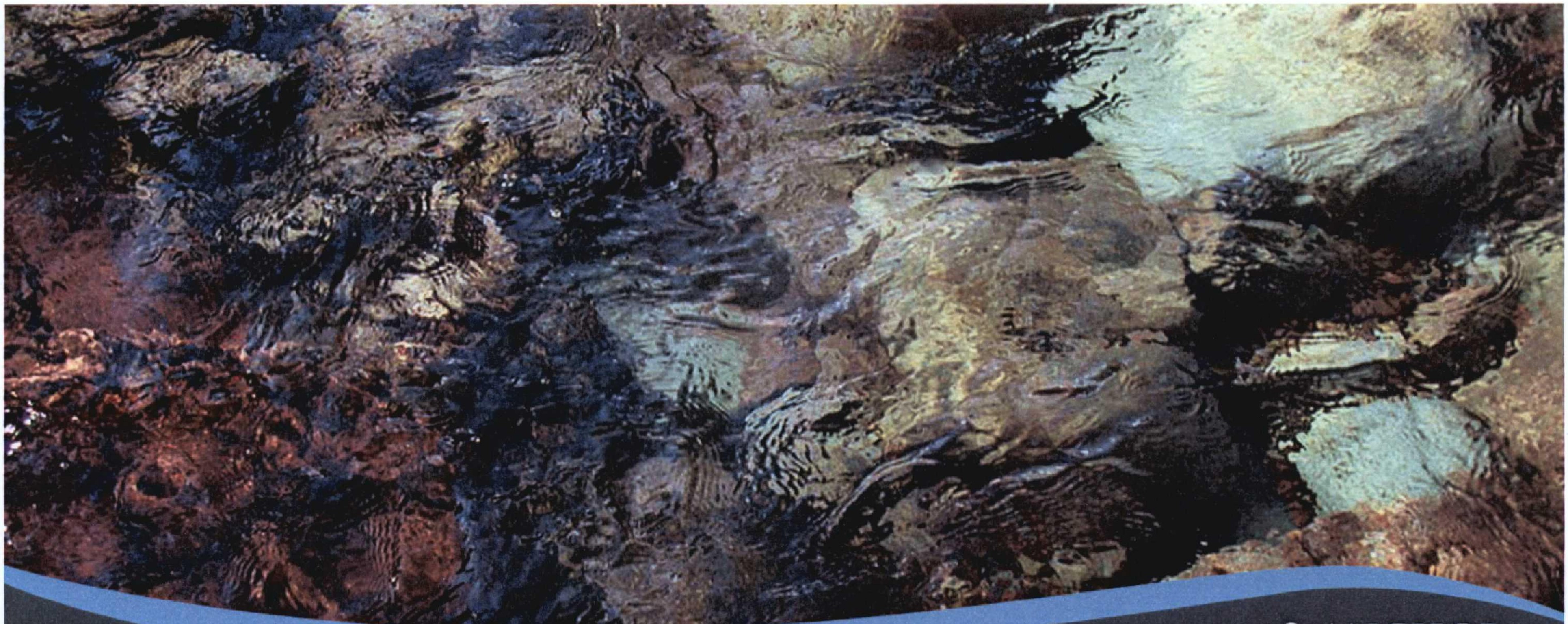
1. Do we have confidence we can prepare and dispense suspensions of LA with known concentration?

2. Do we know the time course of fiber loss in aquaria, with and without fish?

3. Is it possible to design and implement a fish toxicity study that will be credible?

4. If so, what is the design?

5. If not, what altemative options exist for evaluating LA toxicity to fish?



ANCHOR QEA

Libby Superfund Site OU3: Overview of 2011 Pool Temperature Assessment and Pool Size Characterization

Presented by
Joe Volosin

December 8, 2011

Presentation Overview

- OU3, reference site hydrology review
- Purpose of temperature and pool program
- Note on fish barriers
- Overview of pool habitat program
- Methods and findings of pool temperature evaluations
- Methods and findings of pool size characterization

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Introduction

- This study followed the methods outlined in the April 2011 Phase IV Sampling and Analysis Plan (SAP) and Standard Operating Procedures (SOPs) as prepared by the U.S. Environmental Protection Agency (USEPA)

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Locations Evaluated

- The pool habitat assessment was conducted at seven stream locations in OU3, including two in upper Rainy Creek, four in lower Rainy Creek, and one downstream of the tailings impoundment
- Two reference locations in the vicinity of OU3 were also evaluated including one location on a tributary to Bobtail Creek and the other location on Noisy Creek

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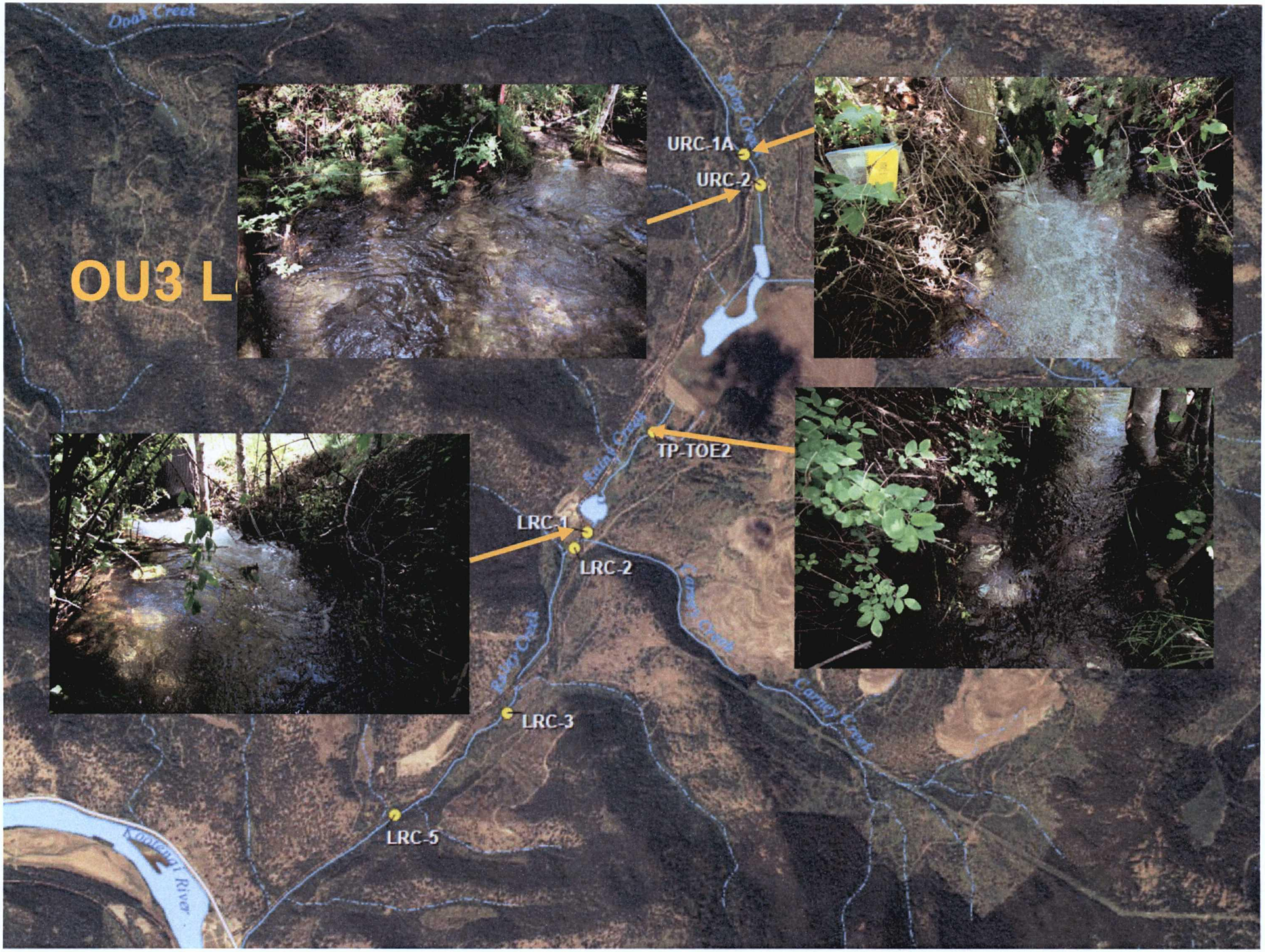
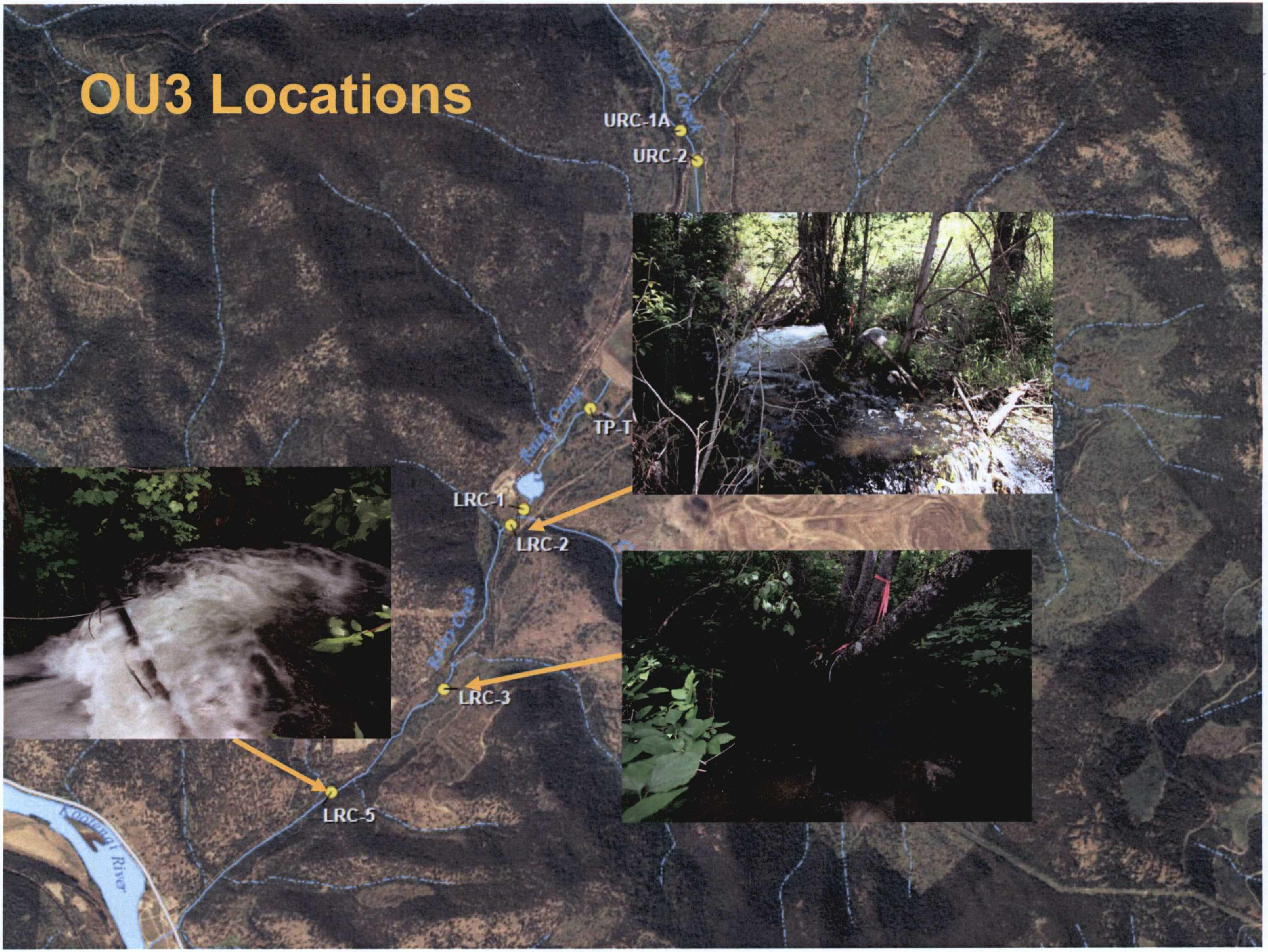

OU3 L
Doak Creek
URC-1A
URC-2
TP-TOE2
Rainy Creek
LRC-1
LRC-2
LRC-3
LRC-5
Carney Creek
Kootenai River

OU3 Locations



ations
NSY-R1
Noisy Creek
South Fork Bristow Creek
Bristow Creek
Hickey Creek
Blue Creek
Barron Creek
Dad Creek
Quartz Creek
Bobtail Creek
Jackson Creek
Little Jackson Creek
Carney Creek
Peace Creek
Alexander Creek
Rainy Creek
Kootenai River
Cedar Creek
Lost Creek
Parmenter Creek
Libby Creek

Purpose of Temperature and Pool Program

- Provide pool size information for HSI model
- The Habitat Suitability Index (HSI) model is being used to evaluate the suitability of Rainy Creek to support and sustain fish populations
- Evaluate differences in stream temperature between locations

OU3, Reference Site Hydrology Review

- Hydrology of some sites is affected by ponds
- The hydrology of upper Rainy Creek is not affected by any ponds/impoundments
- The tailings pond only affects lower Rainy Creek during overflow events
- The mill pond affects lower Rainy Creek
- The hydrology of Bobtail Creek is affected by a pond

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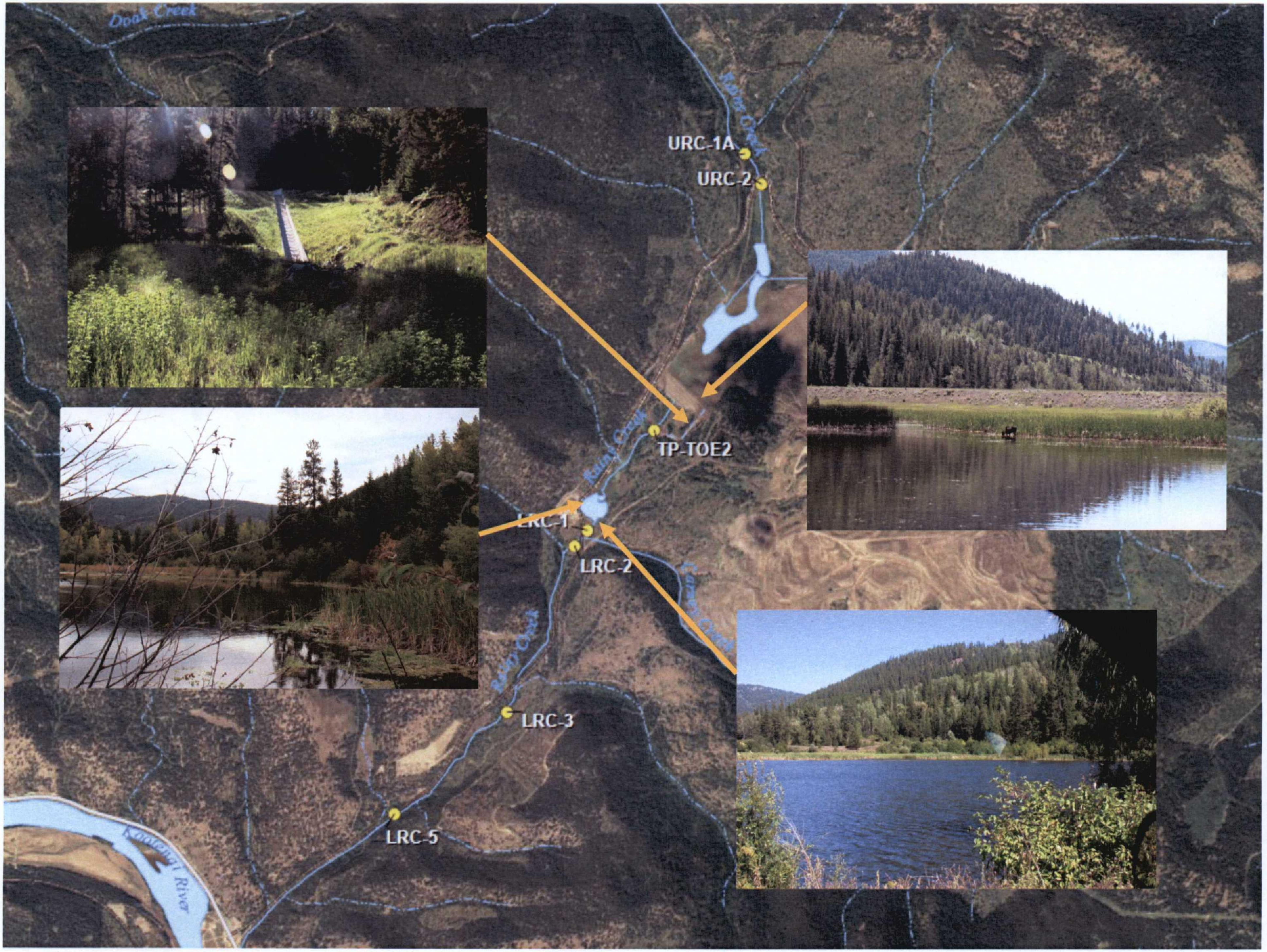
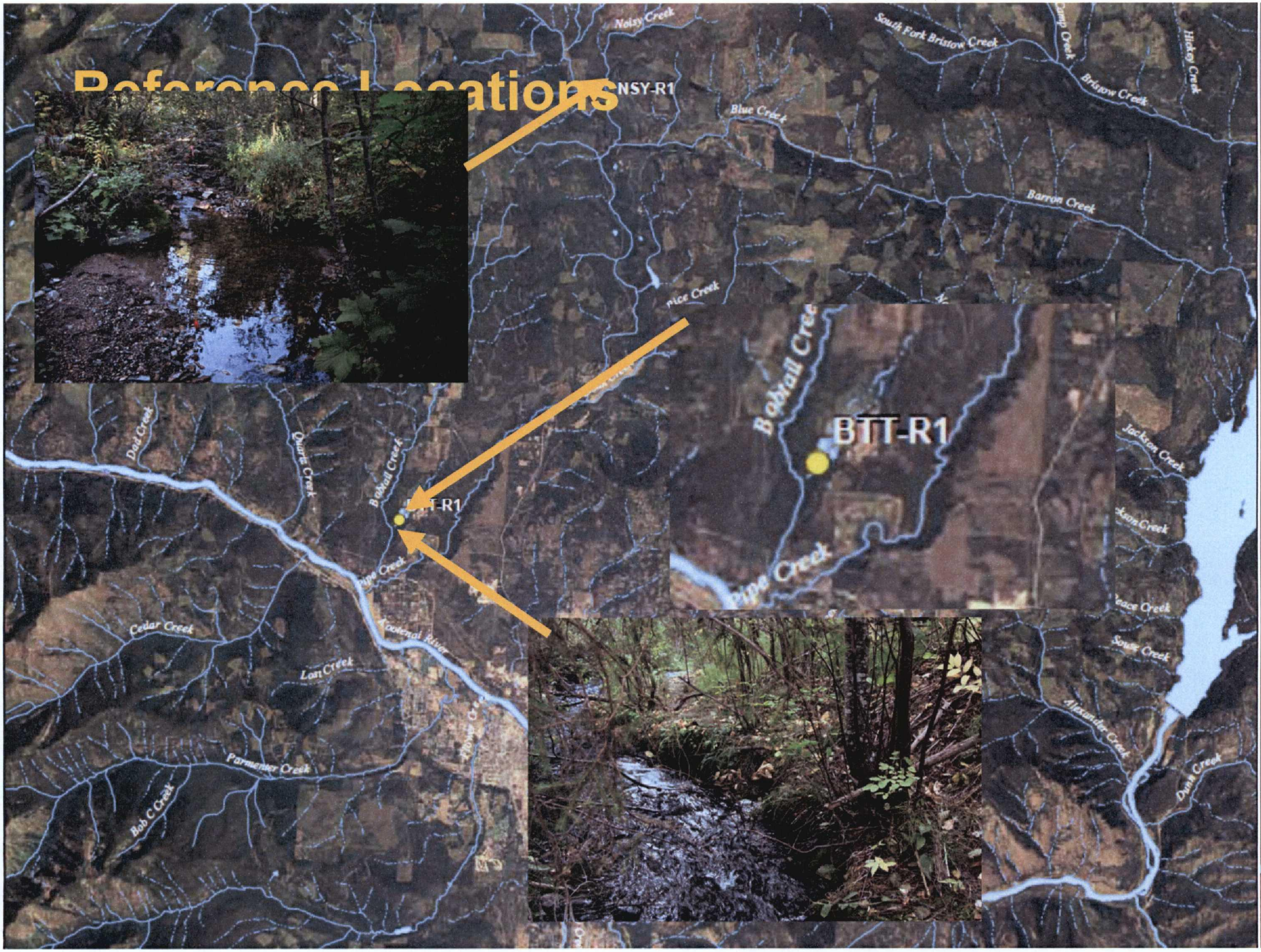


Doak Creek
URC-1A
URC-2
TP-TOE2
LRC-1
LRC-2
LRC-3
LRC-5
Kootenai River


Noisy Creek
South Fork Bristow Creek
Camp Creek
Hickey Creek
Bristow Creek
NSY-R1
Blue Creek
Barron Creek
Bobtail Creek
BTT-R1
Pipe Creek
Dad Creek
Quartz Creek
Jackson Creek
Peace Creek
Souse Creek
Alexander Creek
Dunn Creek
Cedar Creek
Kootenai River
Lost Creek
Parmenter Creek
Bob C Creek

Note on Fish Barriers

- There was one culvert, downstream of LRC-5, that will not allow any fish to pass
- Due to the barrier in lower Rainy Creek, the fish found in lower Rainy Creek are a self-sustained population
- There is no connection between upper and lower Rainy Creek except from the toe drains and the overflow

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OU3 Barrier Locations

Pool Temperature Evaluation

- Pool temperature evaluation approach
- Presentation of results

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Pool Temperature Evaluation Methods

- The pool temperature assessment was conducted by placing a data logger in the deepest pool and deepest point in each pool at each location
- The pool temperature assessment was conducted from June 22 to October 4, 2011
- Pool temperature data were evaluated to assess if loggers were exposed to air; outlier evaluation
- Data were evaluated using basic statistics and plots

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Outlier Evaluation

- Data were plotted on an hourly basis for all locations
- Data were flagged as a potential outlier where a rate of temperature change greater than 3°C per hour was observed (Dunham et al. 2005)
- Data were also flagged when there was a daily mean change of greater than 3°C between 2 successive days

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Outlier Evaluation

- Based on the hourly data plots, there are no obvious changes in temperature, higher or lower, except at reference site BTT-R1
- There were no differences in temperatures equal to or greater than 3 °C between successive 1-hour comparisons of data
- There was only one change in temperature between days that was greater than 3 °C, this occurred at BTT-R1

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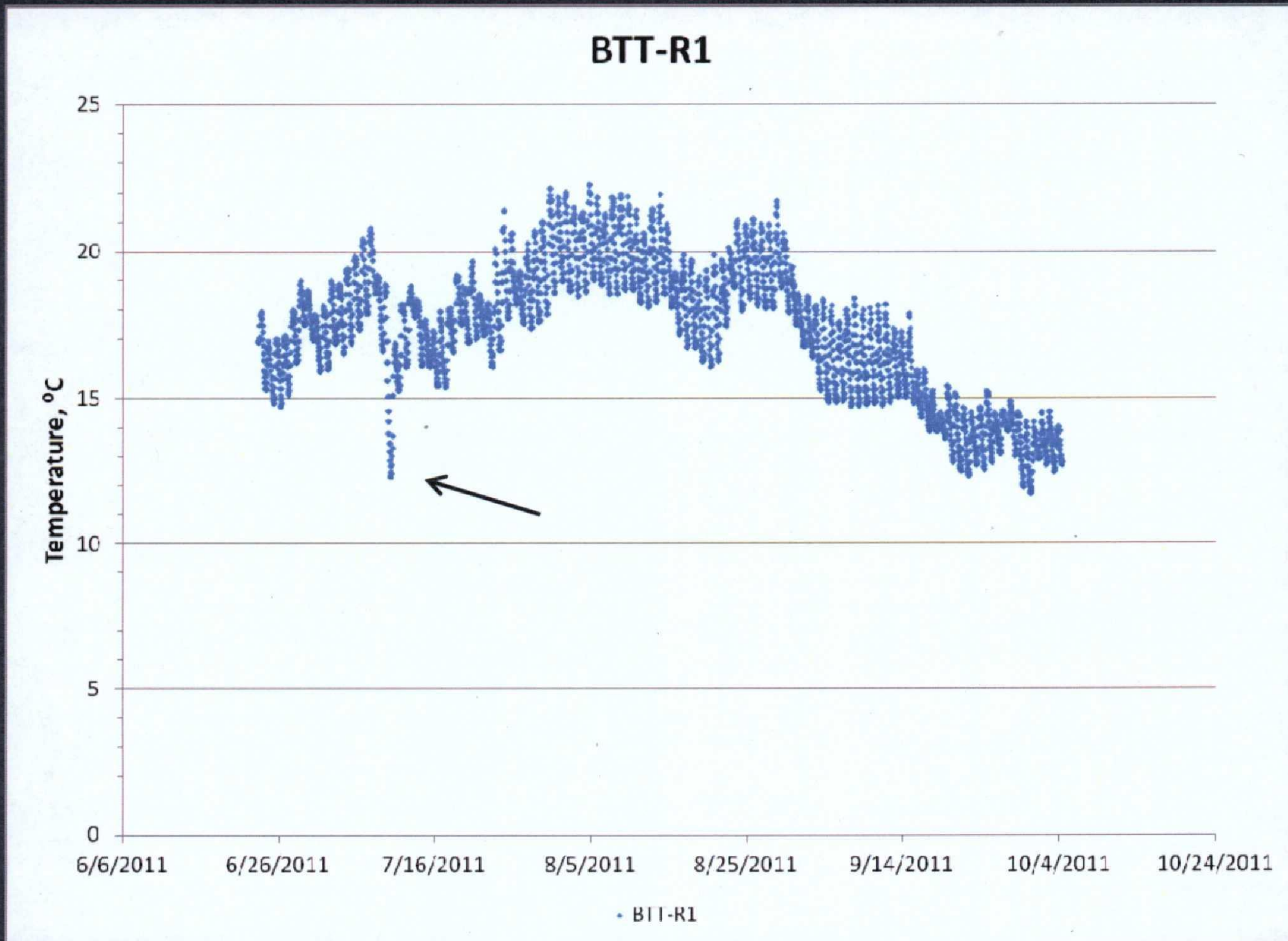
Plot of Hourly Temperature at BTT-R1



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Pool Temperature Assessment Results

- The maximum measured in NSY-R1 was lower than the average measured at BTT-R1
- The upstream sites in the Libby OU3 are clearly cooler than the downstream locations
- The maximum temperatures at URC-1A and URC-2 were lower than average temperatures at LRC-1, LRC-2, LRC-3, and LRC-5

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Summary Statistics

Site ID	Maximum Temperature (°C)	Minimum Temperature (°C)	Average Temperature (°C)	Number of Temperature Observations
NSY-R1	14.1	6.1	10.5	2475
URC-1A	10.4	6.0	8.4	2493
URC-2	10.7	5.7	8.6	2493
TP-TOE2	10.7	8.8	9.8	2492
BTT-R1	22.3	11.7	17.4	2475
LRC-1	20.2	10.2	15.1	2495
LRC-2	20.0	10.0	15.0	2495
LRC-3	17.9	7.9	13.8	2495
LRC-5	17.6	7.1	13.4	2495

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Pool Temperature Assessment Results

- Plots of daily averages for each location illustrate the range of temperatures during the monitoring period
- None of the cooler sites have any influence from a pond or other impoundment
- The warmest locations do have influence from a pond or impoundment

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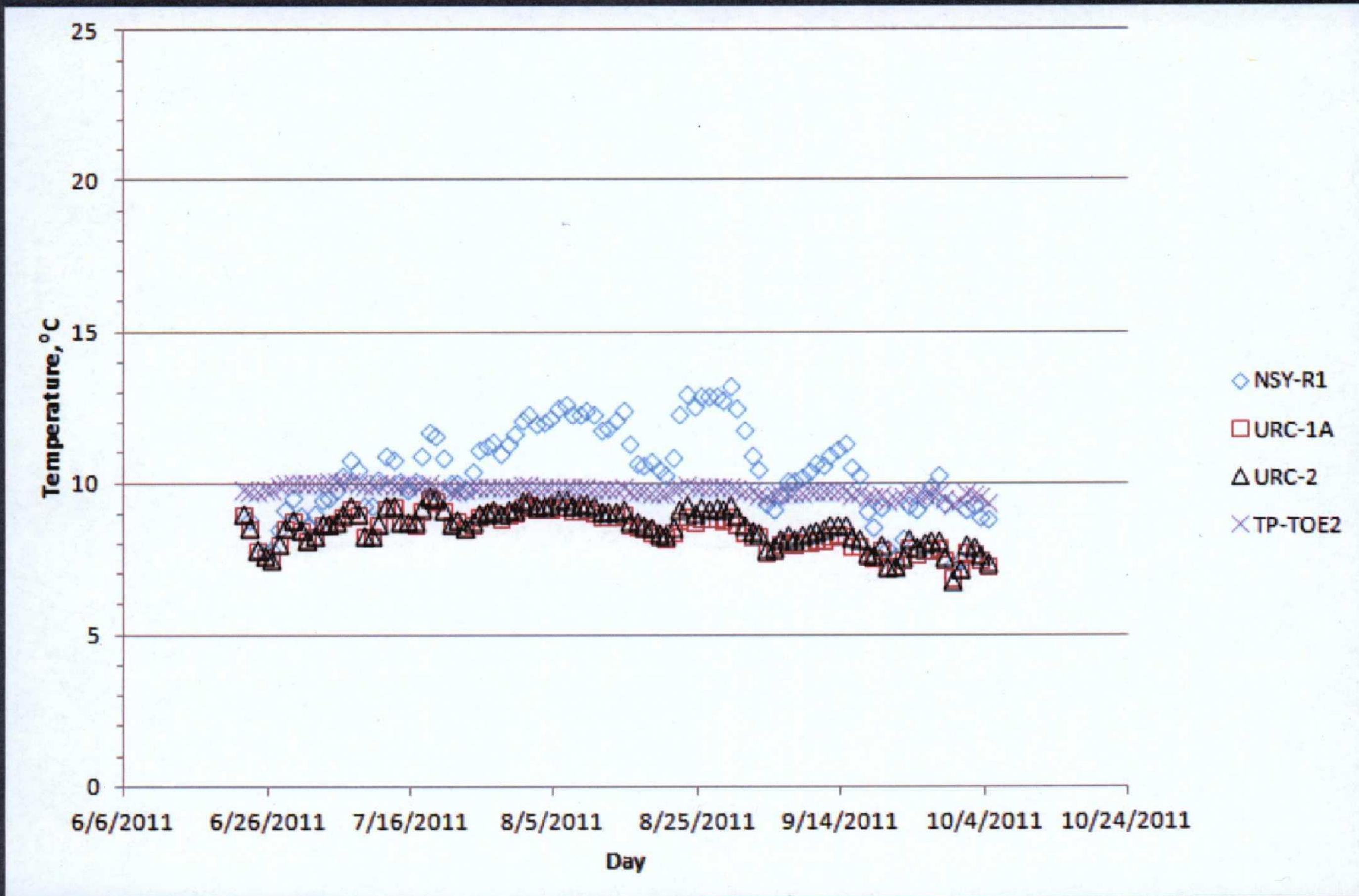
Plots of Daily Averages Temperature at NSY-R1, URC-1A, URC-2, and TP-TOE2



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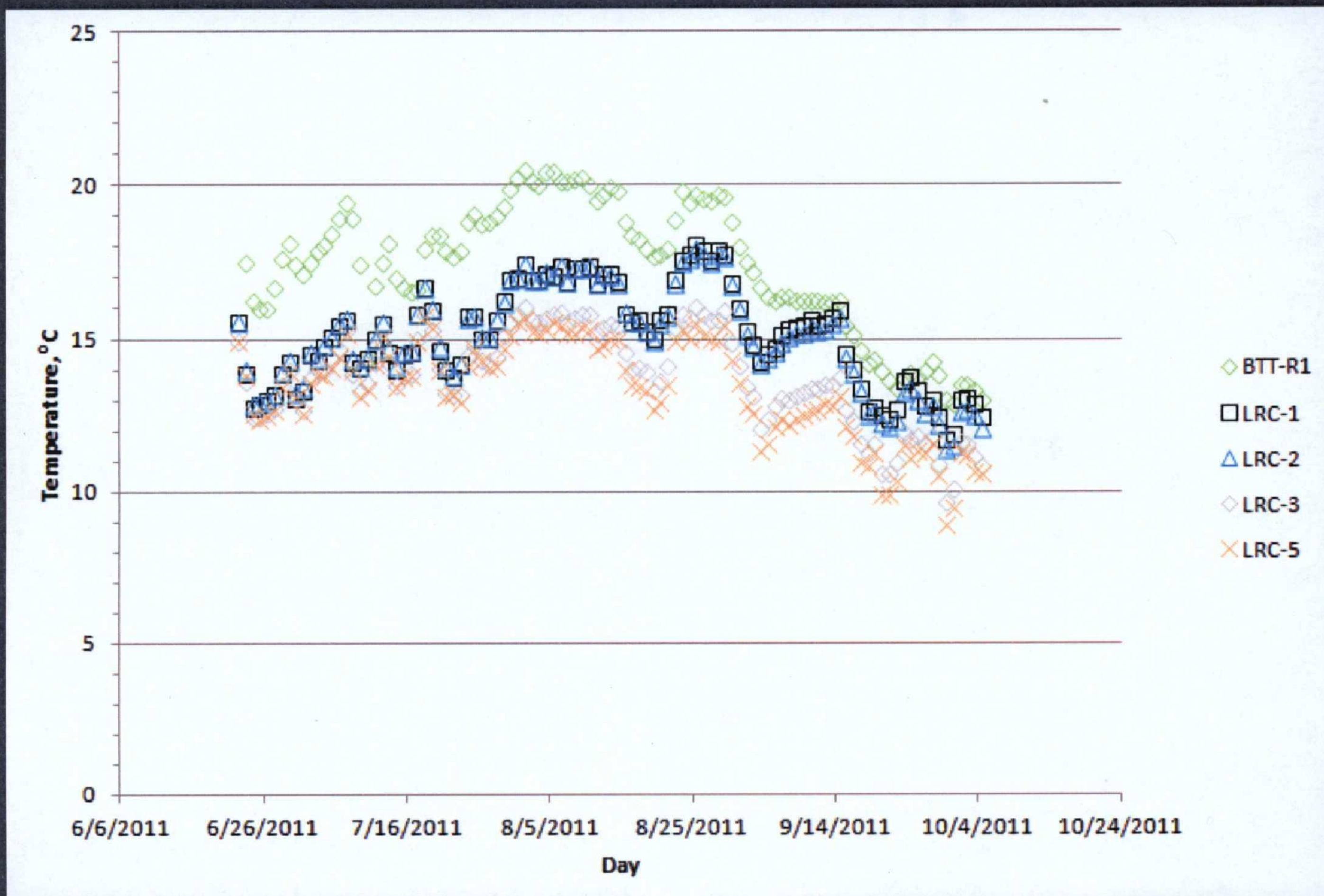
Plots of Daily Averages Temperature at BTT-R1, LRC-1, LRC-2, LRC-3, and LRC-5



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Pool Temperature Data Recap

- The upstream sites in the Libby OU3 are clearly cooler than the downstream locations
- A plot of daily averages for all locations illustrates the range of temperatures during the monitoring period
- None of the cooler water sites have any influence from a pond or other impoundment

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Pool Size Assessment

- Pool size characterization approach
- Pool size classes
- Presentation of results

Pool Size Characterization Methods

- The pool size characterization was conducted in September 2011
- The methods used to conduct the pool size assessment included measuring pool length, pool width at its widest point, and pool depth in the thalweg (deepest point)

Pool Size Class Descriptions

Pool Class	Description
1	Large and deep. More than 30 percent of the pool bottom is obscured due to depth, surface turbulence, or the presence of structures. The pool depth is greater than 1.0 meter deep (in streams less than 5 meters wide).
2	Moderate size and depth. From 5 to 30 percent of the pool bottom is obscured. Typical class 2 pools are large eddies behind boulders and low velocity, moderately deep areas beneath overhanging banks. Pool depth may range from 0.3 to less than 1.0 meter.
3	Small or shallow or both. Cover, if present, is in the form of shade, surface turbulence, or very limited structure. Class 3 pools are wide, shallow pool areas of streams or small eddies behind boulders. The entire bottom is viewable. Pool depth is less than 0.3 meters to 0.2 meters.

Descriptions are from Final Phase IV SAP (April 2011)

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Pool Size Characterization Results

- Only reference site NSY-R1 had a class 1 pool
- The upper Rainy Creek site, URC-1A, had the most area covered by pools
- For the cooler locations, URC-1A had the most pool area covered by class 2 pools followed by TP-TOE2, NSY-R1, and URC-2
- Reference site NSY-R1 had the most area covered by class 3 pools followed by URC-1A, URC-2, and TP-TOE2

Summary of Stream Depths, Widths, Lengths, and Areas

Site ID	Average Depth in Reach (meters)	Average Width in Reach (meters)	Reach Length (meters)	Area (square meters)
URC-1A	0.092	1.56	52.4	82
NSY-R1	0.074	2.02	114	230
URC-2	0.082	2.28	84	192
TP-TOE2	0.162	1.88	97.3	183
BTT-R1	0.144	1.26	82	103
LRC-3	0.262	1.58	64	101
LRC-2	0.19	1.68	103	173
LRC-1	0.194	1.96	85	167
LRC-5	0.14	2.22	66	147

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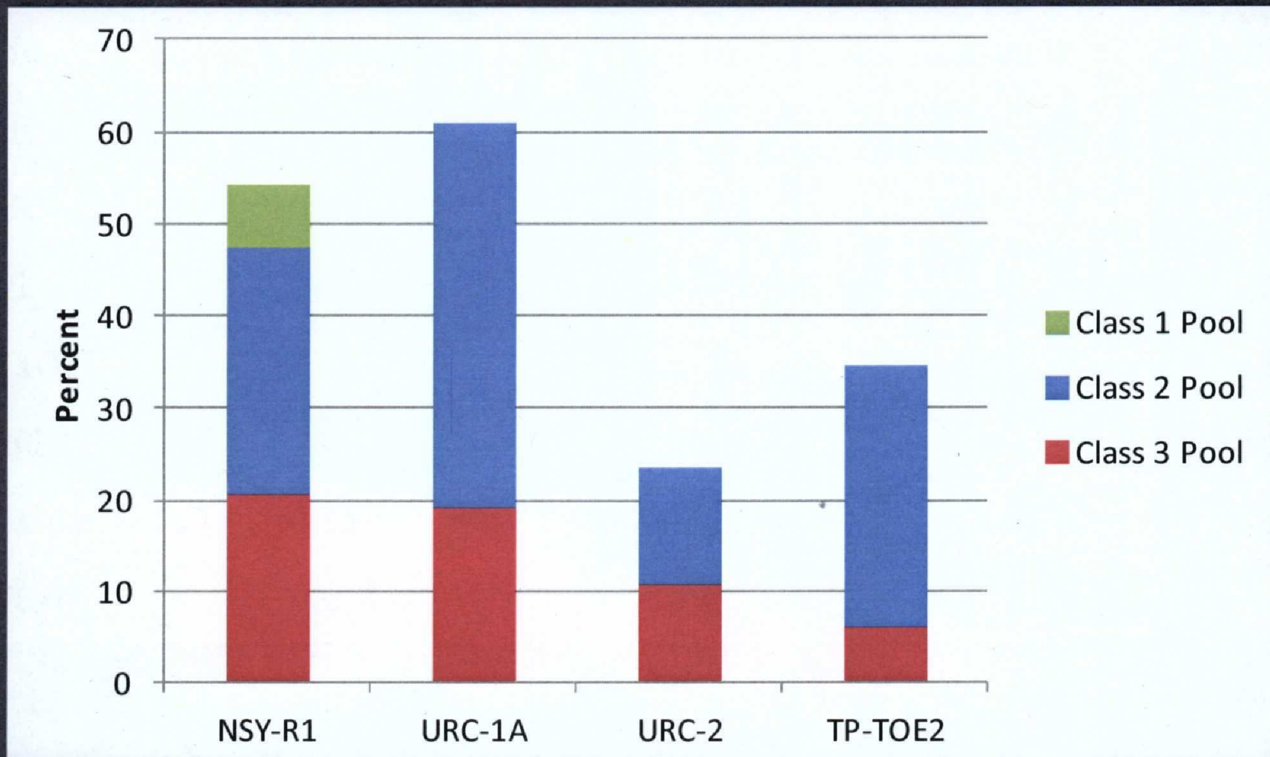
Pool Area in Percent at NSY-R1, URC-1A, URC-2, and TP-TOE2



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Pool Characterization Results

- Reference site BTT-R1 had the least amount of area covered by pools
- For the warmer sites, LRC-3 had the most area covered by class 2 pools followed by LRC-2, LRC-1, LRC-5, and BTT-R1
- The lower Rainy Creek site, LRC-1, had the most area covered by class 3 pools followed by LRC-5, BTT-R1, LRC-2, and LRC-3

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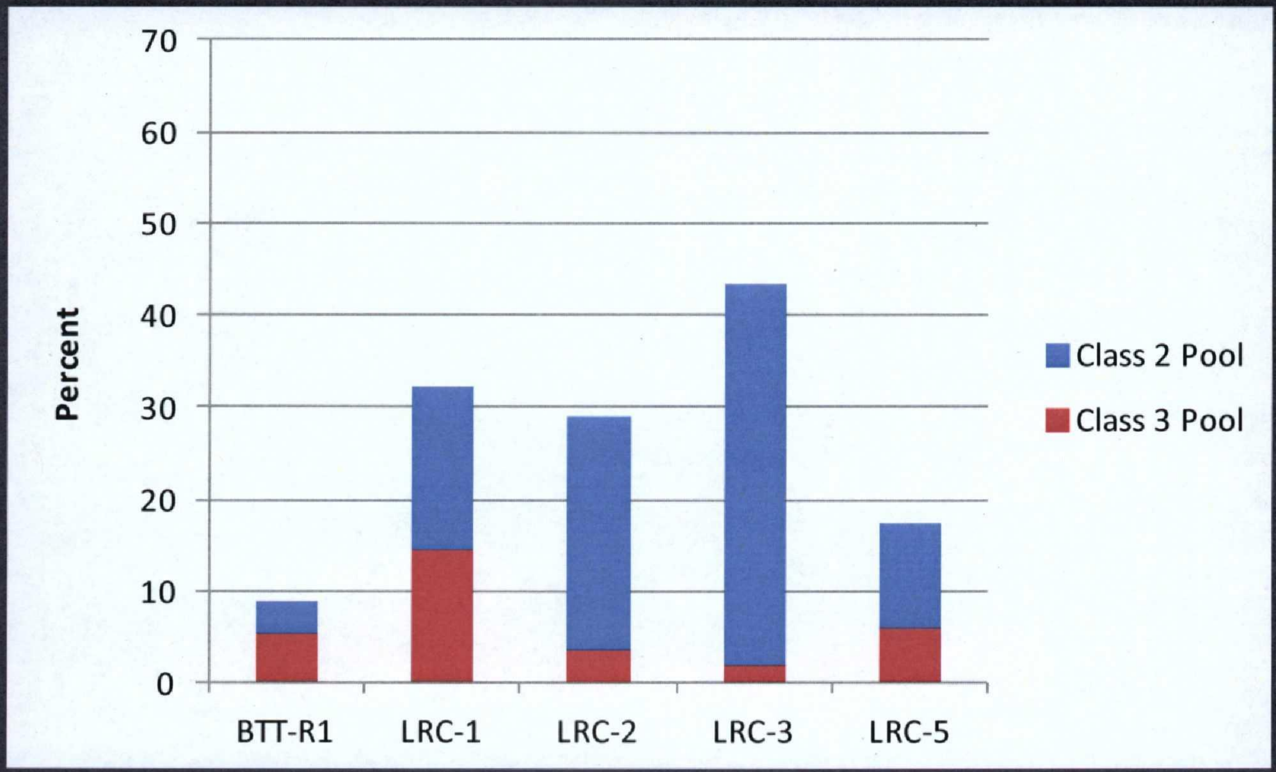
Pool Area in Percent at BTT-R1, LRC-1, LRC-2, LRC-3, and LRC-5



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Pool Size Data Recap

- Only reference site NSY-R1 had a class 1 pool
- Reference site BTT-R1 had the least amount of area covered by pools
- The upper Rainy Creek site, URC-1A, had the most area covered by pools

Conclusions

- There are clear differences in stream pool temperatures when comparing the different stream locations
- The streams not influenced by a pond or impoundment are cooler and the stream locations affected by a pond are clearly warmer
- Reference site NSY-R1 is not affected by a pond, nor are URC-1A, URC-2, and TP-TOE2

Conclusions (cont.)

- Reference site BTT-R1 is much warmer than reference site NSY-R1
- The upper Rainy Creek locations are cooler than the downstream locations
- Differences between sampling locations were found for pool size

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Conclusions (cont.)

- Only one site, NSY-R1, had class 1 pools
- Most locations were dominated by class 2 pools
- Reference site BTT-R1 had the least amount of area covered by pools
- The upper Rainy Creek site, URC-1A, had the most area covered by pools

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AVIAN RESPIRATORY SYSTEM:
Overview of Anatomy and Function as Related to Particulate Inhalation

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INTRODUCTION

The avian respiratory system performs the following fimctions: gas exchange; thermoregulation; phonation; olfaction; air filtration/cleansing; blood filtration; regulation of acid-base balance; and, production and metabolism of blood-bome molecules. This summary will focus first on the macroscopic and microscopic anatomy of the extra- and intra-pulmonary airways and their connections to the air sacs. Pattems of air flow during inspiration and expiration then can be summarized. Finally the defense mechanisms that protect die respiratory system from inhaled particulates and the evidence pertinent to avian particulate inhalation will be reviewed. Extensive reviews of avian respiratory structure and fimction have been published elsewhere (Jukes, 1971; King and Molony, 1971; Duncker, 1974; Nickel et al., 1977; McLelland and Molony, 1983; King and McLelland, 1984; Fedde, 1986, 1998; Brackenbury, 1987; Scheid and Piiper, 1987; King, 1993; Brown et al., 1997). Animated images of air flow pattems through the lungs and air sacs can be found at: http://people.eku.edu/ritchisong/birdrespiration.html. The descriptions contained in the present overview pertain primarily to the respiratory system of the domestic fowl.

ANATOMY

Nasal Passages: Depending on the species, the extemal nasal apertures (nares) at the base of the upper beak may be protected by opercula (partial or complete flaps) or cere and ricti (ridges of skin). Feathers arising from the cere may cover the nares. The nasal cavities contain turbinate bodies consisting of convoluted mucosa-covered cartilage. The nasal cavities open through the choana (medial fissure in the "hard" palate) into the pharynx (common passageway for food, water and air). The slit-like glottis guards the opening from the pharynx into the larynx, and prevents non-aerosol foreign matter (e.g., food and water) from entering the trachea.

Conducting Airways: the trachea conducts air into the thoracic cavity and bifurcates at the syrinx (the avian organ of phonation) to form the right and left extrapulmonary primary bronchi. These bronchi penetrate the respective lungs to become the intrapulmonary primary bronchi (Figure 1). The conducting airways up to this point are reinforced externally with cartilage rings that maintain flexibility while preventing airway collapse. The unilobar lungs are located lateral to the vertebral column in the dorsal thorax. The dorsal-lateral border of each lung interdigitates between 5 ribs, thus approximately 25% of the total lung volume is encased between the ribs (Figures 2 and 3). Within the lungs of domestic fowl, the medioventral (4 each), mediodorsal (8 each), lateroventral (8 each), and laterodorsal secondary bronchi (23-30 each) branch from the intrapulmonary primary bronchus (Figures 1 and 2). These secondary bronchi are not supported by extemal cartilage rings.

Gas Exchange Airways: Arching between the medioventral and mediodorsal secondary bronchi, arcades of long cylindrical paleopulmonic parabronchi (tertiary bronchi) (Figures 2 and 4) are layered adjacent to one another in a roughly hexagonal array (when viewed in cross section; Steams et al., 1987). Individual parabronchi are separated from each other by a thin interparabronchial connective tissue septum containing interparabronchial arteries and veins (Figures 5 and 6). Approximately 500 paleopulmonic parabronchi are found in each lung of domestic fowl. They measure up to 4 cm long, have a uniform outside diameter of 1.5-2 mm and a lumen diameter of 0.5 mm. Between 100 and 300 freely anastomosing neopulmonic parabronchi connect the lateroventral and laterodorsal secondary bronchi (Figure 4). Neopulmonic parabronchi measme up to 1 cm long and comprise 20-25% of the total parabronchial volume.

A simple squamous epithelium lines the parabronchial lumen, but this epithelium is not the site of gas exchange. Instead, as shown in Figures 5 and 6 thousands of atria 100-200µm in diameter form pockets projecting 50µm into the luminal wall. The epithelial cells lining the atria produce surfactant, which coats the inner surfaces of conducting airways and gas exchange membranes. Spiral bands of innervated smooth muscle underlie the parabronchial luminal epithelium and encircle the opening to each atrium (atrial muscle, Figure 6). Elastic fibers encase the walls (septa) and floor of the atria, presumably serving a support function. One or more fimnel-shaped infundibula penetrate from the atrial floor into the parabronchial wall, with multiple freely anastomosing air capillaries originating from each infiuidibulum (Figures 5 and 6). The air capillaries average 8 to 15 µm in diameter and penetrate outward from the infimdibulum, extending 200-500 µm to the outer periphery of the parabronchial wall adjacent to the interparabronchial septum (Figure 6). Each air capillary is surrounded by a profusion of blood capillaries derived from intraparabronchial arterioles that branch inward into the parabronchial wall from the interparabronchial arteries. Gas exchange occurs at the blood-gas barrier, at the interface between blood capillaries and air capillaries (Figure 7).

Air Sacs:Air enters and exits the air sacs via ostea that connect with the intrapulmonary primary bronchi, branches of the secondary bronchi, and terminal neopulmonic parabronchi (Figures 1 and 2). Domestic fowl possess eight air sacs, including one clavicular, one cervical, two cranial thoracic, two caudal thoracic, and two abdominal sacs (Figures 1 and 3). The thin, transparent nonstratified squamous epithelium of the air sacs is poorly vascularized and plays essentially no role in the gas exchange process. The air sac membrane contains small islands of ciliated and secretory cells, and is supported by diffiise elastin fibers (McLelland, 1989). Functionally, the air sacs serve as elastic, inflatable intemal reservoirs for "fresh" and "stale" air. In conjunction with the thoracic and abdominal musculature, the air sacs also act in a bellows-like fashion to propel air through the parabronchi. The extensive penetration of air sacs throughout the thorax, abdomen and skeleton accounts for serious concems regarding carcass contamination that arise when air sacculitis is detected during inspection of poultry at processing plants (King and McLelland, 1984). To simplify further discussion, it is convenient to group the clavicular, cervical and cranial thoracic sacs in the category of cranial air sacs, and the caudal thoracic and abdominal sacs in the category of caudal air sacs.

AIR FLOW DURING INSPIRATION AND EXPIRATION

Avian lungs remain essentially fixed in volume throughout the respiratory cycle, and thus the lungs neither appreciably inflate during inspiration nor deflate during expiration. The current consensus is that all intrapulmonary air channels remain open and relatively fixed in volume throughout the respiratory cycle. Consequently, air must be forced to flow through the intrapulmonary conducting airways by the bellows-like action of the air sacs. A saccopleural membrane is anchored by skeletal muscle (costoseptal muscle) to the intemal thoracic wall and covers the ventral lung surface. This membranous structure is penetrated by the ostea to the caudal air sacs and, unlike the mammalian diaphragm, the avian saccopleural membrane does not contribute to the development of a negative intrathoracic pressure. The costoseptal muscles apparently contract during expiration to hold the ostea open (King and McLelland, 1984). Thus birds lack a fimctional diaphragm and must depend entirely on the contraction and relaxation of thoracic and abdominal muscles during inspiration and expiration.

During inspiration the rib cage and sternum expand to more cranial and ventral positions, increasing the thoracic volume and generating a negative intrathoracic pressure (suction). Simultaneous relaxation of the abdominal muscles coupled with the forward excursion of the sternum and gravitational pull on the visceral organs increases the volume of the abdominal cavity. The resulting negative thoraco-abdominal pressures (-1 cm H_2O) serve to inflate (draw air into) the cranial and caudal air sacs simultaneously (**Figure 8**, upper panel). "Fresh" air enters the trachea and is drawn through the extra- and intra-puhnonary primary bronchi toward the caudal air sacs. This incoming air does not enter the medioventral secondary parabronchi due to their acute caudally-directed angle of insertion along the intrapulmonary primary bronchus. Instead, the incoming fresh air is drawn caudally to: (a) mix with and carry end expiratory stale air from the trachea and primary bronchus, through the neopuhnonic parabronchi and into the caudal air sacs; (b) supply the neopulmonic parabronchi and caudal air sacs with fresh air; and, (c) flow through the mediodorsal secondary bronchi, pushing the resident stale air out of the paleopulmonic parabronchi, through the medioventral secondary bronchi and into the cranial air sacs. Thus the caudal air sacs are inflated mainly with fresh air, and the cranial air sacs are inflated mainly with stale air from the paleopulmonic parabronchi (**Figure 8**, upper panel). Throughout the respiratory cycle, ongoing gas exchange occurs between the blood capillaries and air capillaries. Consequently, with the cessation of fresh air inflow at the end of inspiration, parabronchial air once again becomes stale (PCO_2 increases, PO_2 decreases).

During expiration the rib cage and sternum are drawn inward to more caudal and dorsal positions, reducing the thoracic volume and generating a positive intrathoracic pressure. Simultaneous contractions of the abdominal wall muscles reduce the volume of the abdominal cavity. The resuhing positive thoraco-abdominal pressures (+1 cm H_2O) *partially* deflate the cranial and caudal air sacs (**Figure 8**, lower panel). The stale air from the cranial air sacs flows through the medioventral secondary bronchi, into the primary bronchus and then cranially out through the trachea. The relatively fresh air in the caudal air sacs is forced cranially, and due to aerodynamic valving most of the air exiting the caudal air sacs first perfuses the neopulmonic parabronchi and then flows through the mediodorsal secondary bronchi. After entering the mediodorsal secondary bronchi, the relatively fresh air flows through the paleopulmonic parabronchi. The stale air that is displaced from the paleopuhnonic parabronchi flows, along with stale air from the cranial air sacs, through the medioventral secondary bronchi into the primary bronchus and out through the trachea (Figure 8, lower panel). Aerodynamic valving within the conducting airways insures that the cranial air sacs always serve as a reservoir for *stale* air exiting the parabronchi during inspiration, whereas the caudal air sacs mainly serve as a reservoir for *fresh* air to supply the parabronchi during expiration. This flow of "fresh" air during inspiration and expiration always is unidirectional in the paleopulmonic parabronchi (mediodorsal secondary bronchus to medioventral secondary bronchus), but is bidirectional in die neopulmonic parabronchi (e.g., air flow cessation and reversal occur in the neopulmonic parabronchi during each respiratory cycle, as well as in all air sacs).

As shown in Figures 6 and 7, each parabronchus can be modeled as a long tube with air capillaries (resembling the bristles of a bottle brush) radiating outward at right angles from the parabronchial lumen. During inspiration and expiration, rapid convective air flow occurs along the lumen of the parabronchus. Convective air flow may carry air as deep as the infimdibula (Steams et al., 1987). However, O_2 must move through the gas exchange region of the parabronchus by the relatively slow process of diffusion from the infundibulum to the periphery of the air capillaries, across the blood-gas barrier[1], through the plasma, and into the red blood cells (Powell, 1982; Scheid and Piiper, 1987). Blood capillaries carry deoxygenated blood inward (convective blood flow) following the air capillaries back to their junction with the infundibulum near the parabronchus lumen. Because convective air flow occurs longitudinally down the lumen of the parabronchus, whereas blood flow and gas exchange occur in a transverse path across the radius of the parabronchial wall, the patteri of blood flow and air flow in avian lungs has been labeled a cross-current exchange system. When compared with mammalian respiratory systems, the cross-current avian respiratory system pennits a higher degree of removal of O_2 from respiratory air, and provides exceptional advantages at low atmospheric pressure (low PO_2), as confumed by the exceptional tolerance of birds to high ahitude. Sparrows are able to fly at an atmospheric pressure of 349 mmHg, corresponding to an altitude of 6100 m, while mice are comatose and nearly unable to crawl under identical conditions (Schmidt-Nielsen, 1975).

RESPIRATORY SYSTEM DEFENSES

Nasal Passages: Feathers covering the nares serve to coarsely filter the incoming air. Turbulent air flow within the nasal passageways forces the inhaled air to swirl over the mucosal surfaces of the turbinate bodies. The air becomes humidified (fully saturated with water vapor), wanned to the bird's body temperature, and cleansed of larger particulates that adhere to the mucus. Additional particulate entrapment is likely to occur as the inhaled air flows through the moist, narrow choanal slit in the hard palate and flows over the moist surfaces of the pharynx and glottis (Hayter and Besch, 1974; Fedde, 1998; Brown et al., 1997).

Conducting Airways: The avian trachea, primary bronchi, and initial roots of secondary bronchi are lined with a mucociliary epithelium (a pseudosfratified, longitudinally folded ciliated epithelium with mucous-secreting goblet cells). Pathogens and airbome particles become trapped

[1] The blood-gas barrier is composed of the blood capillary endothelium and its basal lamina, the thin air capillary epithelium, and a thin layer of surfactant. In chickens, the endothelium comprises 67% of the barrier thickness, die basal lamina comprises 21%, and the epithelium plus surfactant comprise only 12% of the ban-ier thickness.

in the mucus, and ciliary action sweeps the mucous cranially (at a rate of 10 mm/min; Fedde, 1998) to the oral cavity where it is swallowed or expectorated (King and Molony, 1971; King and McLelland, 1984). In addition to mucus, the fluids lining avian conducting airways contain antioxidants and surfactant binding proteins that assist in binding and neutralizing inhaled pathogens and antigens (Bottje et al., 1998; Zeng et al., 1998; Johnston et al., 2000). When mammals and birds of similar sizes are compared, the avian trachea is approximately 2.7X longer and has a I.3X larger radius, which yields a 4X greater tracheal volume. (King and McLelland, 1984). Accordingly, the mucociliary escalator has a substantially enhanced opportunity to trap pathogens and particulates in birds when compared with mammals. The mucociliary escalator is an active and highly important line of defense in birds, preventing many aerosol particulates and pathogens from entering the gas exchange parenchyma. For example, poultry reared on floor litter are chronically challenged with air-bome dust, bacteria, and potent antigens (Anderson et al., 1966; Hayter and Besch, 1974; Gross, 1990; Whyte, 1993; Brown et al., 1997; Zucker et al., 2000; Bakutis et al., 2004; Lai et al., 2009). Only modest changes in respiratory function can be detected when broiler chickens (meat-type chickens bred for extremely fast growth and breast muscle accretion) reared on floor litter are compared with broilers reared in much cleaner environments (Bottje et al., 1998; Wang et al., 2002; Lorenzoni and Wideman, 2008). Commercial poultry populations reared on floor litter typically grow rapidly, thrive and reproduce while exhibiting minimal mortality levels. Furthermore, necropsies of clinically healthy broilers reared on floor litter overwhehningly reveal healthy tracheas, almost pristine air sacs (e.g., uniformly clear and transparent membranes), and macroscopically unremarkable lungs (Wideman et al., 2011).

In commercial poultry the respiratory system becomes dramatically more susceptible to damage if mucociliary transport is inhibited by exposure to noxious gasses (e.g., ammonia) and pathogens such as infectious bronchitis virus (IBV), infectious laryngotracheitis (ILT), avian influenza (AI), Newcastle disease virus (ND), and *Mycoplasma gallisepticum*. For example, IBV causes ciliostasis and distinctive symptoms of upper airway distress (gasping, coughing, gurgling) attributable to obstruction of the trachea by mucus accumulation. Inhibition of the mucociliary escalator in combination with distressed pattems of breathing apparently allow pathogenic bacteria and aerosolized respirable particles to penetrate more readily into the lung parenchyma and air sacs. The ensuing pulmonary inflammation and air sacculitis (infection of the air sacs) are profoundly deleterious (Gross, 1961, 1990; Tottori et al., 1997; Yamaguchi et al., 2000).

Bronchus-associated lymphoid tissues (BALT) constitutively develop in the bronchial mucosa at the junctions of primary and secondary bronchi, and at the ostea to the air sacs of clinically healthy birds (Reese et al., 2006). BALT contain lymphocytes (B cells and T cells), lymphoid nodules, and epithelial cells. The mucosal BALT tissues may fimctionally compensate for the absence of fully formed lymph nodes in birds, although their specific role remains to be elucidated (Reese et al., 2006).

Gas Exchange Airways and Air Sacs: Whereas the overwhelming majority of airbome particles exceeding 5 μm in diameter are trapped in the nasal cavities and trachea, some of the smaller respirable particles averaging <5 μm in diameter do reach the avian parabronchi and abdominal air sacs (Hayter and Besch, 1974; Mensah and Brain, 1982; Steams et al., 1987; Fulton et al., 1990). Respirable particles can be heavily contaminated with a wide range of immunogenic substances including pathogens and toxins (Bakutis et al., 2004). Macrophages and neutrophils play a central role in the mammalian responses to aerosolized particulates, and intra-alveolar macrophages serve as a first line of defense at mammalian gas exchange surfaces. In contrast, healthy birds do not appear to maintain large populations of resident macrophages or other resident leukocytes at their gas exchange surfaces (air capillaries) or within their air sacs, although some macrophages have been detected in the atria and infimdibula of the parabronchi, as well as in the larger conducting airways (Maina and Cowley, 1998; Nganpiep and Maina, 2002). The primary phagocytic fimction within avian parabronchi apparently resides within the epithelial cells lining the atria and infimdibula (the same cells that secrete surfactant). These phagocytic endothelial cells engulf particles encountered on their luminal (air space) surface. The internalized particles then may be degraded/digested intracellularly, or they undergo exocytosis to the underlying interstitium. There they are engulfed by resident macrophages located in the spaces between the atrial and infimdibular epithelial cells (Steams et al., 1987; Brovm et al., 1997; Reese et al., 2006). Large numbers of macrophages can be induced to enter the air sacs by injecting appropriate antigens or pathogens into the air sac lumen (Fedde, 1998; Reese et al., 2006). During respiratory infection or aspiration of particulates, phagocytic macrophages and heterophils (analogous to mammalian neutrophils) can be found in lavage fluid from the avian respiratory tract, indicating mechanisms do exist that allow substantial populations of phagocytic leukocytes to enter the gas filled spaces when necessary (Ficken et al., 1986; Toth and Siegel, 1986; Toth et al., 1987, 1988; Qureshi et al., 1993; Klika et al., 1996; Lorenzoni et al., 2009; Maina and Cowley, 1998; Nganpiep and Maina, 2002). Intratracheal instillation of C. *parvum* or *E. coli* effectively increased the number of phagocytes collected by lung lavage within 24 h (Toth et al., 1987). Additionally, macrophages have been reported to migrate into air capillaries in a variety of infectious diseases, including toxoplasmisis, fatal viral hydropericardium syndrome, highly pathogenic infectious bursal disease and highly pathogenic avian influenza (Hower, 1985; Abe et al., 1998; Nakamura et al., 2001). Pathways by which macrophages that have engulfed pathogens or foreign particles are cleared from the lung parenchyma and air sacs remain to be elucidated.P hagocytosed materials may be transported and presented to the local BALT, or they may be transported to peripheral lymphoid organs (e.g., the spleen) (Fedde, 1998; Reese et al., 2006).

Vascular Defenses: Blood-borne particulates and antigens also trigger intrapulmonary immune responses. In addition to particles or pathogens entering the blood stream directly, materials engulfed by lymphatic capillaries subsequentiy flow through major lymph trunks that empty into the vena cava. Thus the lungs perform the important fimction of filtering and clearing the retuming venous blood of micro- and macro-particulates including bacteria and thrombi, as well as other potent antigens translocated from pathogens resident in the intestine or from sites of infection (Weidner and Lancaster, 1999). In some mammalian species blood-bome antigens are primarily removed from the blood stream by pulmonary intravascular macrophages (PIMs), which are large mature macrophages bound to the pulmonary capillary endothelium. However, resident PIMs are not present in chickens (Lund et al., 1921; Winkler, 1988; Staub, 1994; Wamer et al., 1994; Brain et al., 1999; Weidner and Lancaster, 1999). The absence of PIMs does not leave chicken's lungs immunologically unresponsive to blood-bome antigens because the entire blood volume and thus all of the circulating leukocytes flow through the lungs (e.g., the lungs receive 100% of the cardiac output via the pulmonary circulation). For example, intravenously injected cellulose microparticles (30µm diameter) become entrapped in inter- and intra-parabronchial pubnonary arterioles of broiler lungs. Within 20 minutes post-injection the microparticles trigger marked pulmonary inflammatory responses, including perivascular infiltration of mononuclear cells in combination with luminal accumulations of macrophages. During the ensuing 48 hours occlusive particles are surroimded by granulomatous tissue consisting primarily of macrophages, giant cells, and fibrous tissue. Subsequently virtually all of the microparticles are cleared from the lungs within approximately 3 weeks post-injection, the inflammatory response subsides, and the lung parenchyma again retums to an entirely normal (e.g., non-inflamed, unobstructed) histological appearance (Wideman et al., 2002, 2007, 2011a,b; Wang et al., 2003; Hamal et al., 2008, 2010). Avian lungs possess an impressive ability to eliminate (digest), clear (remove), or segregate (wall off) offending particulates.

DISTRIBUTION, DEPOSITION AND CLEARANCE OF INHALED PARTICULATES: RELEVANT RESEARCH SYNOPSIS

Peacock and Peacock (1965) injected finely ground asbestos fibers suspended in tributyrin (a triglyceride ester of glycerol and butyric acid) into the clavicular air sacs of aduh White Leghorn chickens. The injected material spread throughout the air sac and entered the lung parenchyma. Immediate responses were inflammatory, with macrophages engulfing the asbestos fibers and clearing them from the air sacs (presumably into sub-epithelial spaces). Neoplastic and granulomatous tumors formed near the site of injection in 4 out of 30 injected birds. The granulomatous tumor contained asbestos fibers. Evidently the majority of injected birds lived for >3 years. Necropsies conducted 4 years post-injection revealed asbestos fibers remaining in the lung parenchyma, and "asbestos bodies" (asbestos fibers engulfed by macrophages or encased in mineralized connective tissue) were indentified in the "interalveolar septa" (presumably the interatrial septa where clusters of resident macrophages have been demonstrated in chickens by Reese et al., 2006).

Hayter and Besch (1974) evaluated the distribution of aerosolized spherical particles in spontaneously breathing adult roosters. Larger particles (≥3.7µm diameter) primarily were deposited in the nasal passageways and cranial segment of the trachea, although a portion of these particles also entered the caudal air sacs. Smaller particles (≤1.1µm diameter) tended to avoid entrapment in the upper afrways and instead were distributed to the lungs and caudal air sacs. Particles were considered to accumulate preferentially at locations where branching of the conducting airways (e.g., rapid amplification of the cumulative luminal cross-sectional area caudal to the syrinx) caused abrupt reductions in air flow velocities, or where reversal of air flow occurred (e.g., in the caudal air sacs) (Hayter and Besch, 1974).

Brambilla et al. (1979) retrospectively evaluated pubnonary lesions in tissues saved during routine necropsies of 11 mammalian and 8 avian species that had chronically inhaled air containing high levels of silicate particles (1 to 10µm in length) while residing at the San Diego Zoo. All of the avian species exhibited severe silicate dust deposition in the tertiary bronchi (parabronchi), accompanied in some individuals by the formation of large granulomas composed of crystal laden macrophages. Fibrosis and necrosis were absent, and none of the birds had been reported to have respiratory problems. Particles deposited in the conducting airways evidently were effectively cleared by mucociliary escalator, whereas those engulfed by parabronchial epithelial cells or macrophages were much more difficult to clear and, consequently, triggered ongoing immunological responses. When compared with mammals, all of the avian species evaluated in this study appeared to be more susceptible to parenchymal silicate dust retention and granuloma formation (birds were less capable of clearing particulates reaching the non-ciliated secondary and tertiary bronchi), but birds were significantly less susceptible to pulmonary fibrosis (Brambilla et al., 1979).

Mensah and Brain (1982) evaluated the deposition and clearance rates for aerosolized particles (< 0.8μm diameter) in unanesthetized spontaneously breathing hens. Particles of this size were only sparsely deposited in the trachea but considerable deposition was detected in both lungs. More particles accumulated in caudal than cranial portions of the lungs, presumably reflecting preferential particle deposition in the neopulmonic parabronchi where air flow velocities decrease and then abruptly reverse direction. Ahnost half of the particles had been cleared from the lungs within 1 hour post-inhalation, and 65% of the particles were cleared from the lungs within 12 hours. This rapid phase of clearance presumably reflects the activity of the mucociliary escalator, which appears to be considerably more vigorous in birds than the more sluggish clearance rate for similarly sized particles deposited in mammalian lungs. As particles were cleared from the lungs they accumulated in the gastrointestinal tract (presumably after the tracheal mucus was swallowed) and were eliminated in the feces. Approximately 35% of the particles persisted in the lung parenchyma through the end of the study (36 hours), presumably reflecting the proportion engulfed by parabronchial epithelial cells and interstitial macrophages. Particles also accumulated in pneumatized bones that are penetrated by cranial air sacs, indicating significant numbers of particles streamlined completely through the paleopuhnonic parabronchi and thus were dispersed into the cervical and clavicular air sacs (Mensah and Brain, 1982).

Nakaue, Pierson and Heifer (1982) and Bland, Nakue, Goeger and Heifer (1985) evaluated the performance and health responses of broiler chickens exposed to Mount St. Helen's volcanic ash (VA; particles ranging from 0.5 to 10 μm diameter). The VA was applied directiy to the wood shavings litter on the pen floor, or was blown daily (for 20 consecutive days) into pens with resident birds.When compare d with unexposed control birds, none of the modes of VA exposure altered any of the routine indices of broiler performance, including final body weights, feed conversion, carcass quality, and cumulative mortality. Litter moisture and ammonia levels also were unaffected by VA, suggesting the absence of significant damage to the kidneys and gastroimtestinal tract. Aerosol induction of VA did not alter the histological appearance of the turbinate bodies or the trachea, but pathological changes within the lungs were detected in a portion of the birds beginning 4 days post-exposure. Macrophages initially phagocytized the VA dust within secondary and tertiary bronchi. More chronically, a mild lymphoid hyperplasia developed, including the formation of granulomas containing giant cells surrounding phagocytized crystalline material (Nakaue et al., 1982; Bland et al., 1985).

Steams et al. (1987) exposed spontaneously breathing adult female ducks to aerosolized iron oxide (0.18μm diameter). The ducks were euthanized 24 hours post-exposure, and transmission electron microscopy was used to evaluate particle deposition within the parabronchial parenchyma. Particle clearance from the parabronchial lumen followed a distinctive sequence:

(a) entrapment in the relatively thick layer of surfactant; (b) phagocytosis by the luminal surface membranes of atrial and infimdibular epithelial cells (the same cells that secrete surfuctant); (c) movement of the phagosome to the basal-lateral surfaces of the epithelial cells; (d) exocytosis of the particles into the interstitial spaces; and, (e) phagocytosis of the particle by atrial and infimdibular interstitial macrophages (macrophages were not seen on the epithelial/luminal surface). The disposition of the particles after their phagocytosis by interstitial macrophages was not assessed. Relatively few particles were observed in the air capillaries per se, leading to the interpretation that aerosolized particles were distributed to the atria and infimdibula primarily by convective air flow (Steams et al., 1987).

Brown et al. (1997) reviewed the structure and fimction of avian respiratory system in relation to its susceptibility to damage by inspired particles and toxins. Deposition pattems for aerosolized particles of different sizes and shapes were predicted based on the anatomy of the airways and the physical forces acting on the particles (e.g., inertial forces, gravitational sedimentation, and Brownian diffusion). Inertial impaction was predicted to clear larger particles primarily in the nasal passageways, pharynx, larynx, trachea, syrinx, and points where secondary bronchi branch from intrapulmonary primary bronchi. Gravitational sedimentation and Brownian diffusion were predicted to occur where air velocities are low and particle residence time is prolonged, particularly within the air sacs and parabronchi (Brown et al., 1997).

SYNTHESIS FROM THE AVAILABLE INFORMATION

I. Particle size distributions for the Libby Amphibole (LA) in duff (Figure 9) indicate that, <u>if suitably aerosolized</u>, well over half of these particles are small enough to be distributed throughout the avian respiratory system, including to the level of the parabronchial atria and infimdibula.

- Ground foraging birds are likely to stir up the duff and kick LA particles into the air; the worst case scenario is created by dust-bathing bh'ds.
- The LA particles may not be easily aerosolized during foraging or dust bathing, but some of the smallest particles may adhere to other inspirable "dust" that more readily becomes suspended as a colloid in the air when the duff is disturbed.

2. Over a period of months or years some of the LA particles are likely to be inspired by ground dwelling/foraging birds.

- Particles trapped in the protective mucus of the nasal passageways, pharynx and ciliated conducting airways will have little biological impact on those structures, and will be cleared rapidly by the mucociliary escalator. Mucus containing particles cleared from the upper airways will be swallowed, enter the gastrointestinal tract, and excreted in the feces. Evaluation of LA content within the core matrix of avian fecal pellets collected within the zone of contamination may constitute the simplest way to directly quantify the possibility that a threat exists.
- Particles deposited in the parabronchi will be phagocytized predominately by epithelial cells that line the atria and infimdibula, but also by resident macrophages in the lumen and interstitial macrophages. Engulfed particulates composed of substances that cannot be degraded or digested intracellularly by the epithelial cells and interstitial macrophages

appear to pose a specific problem for birds: the epithelial cells (and apparently the interstitial macrophages) remain *in situ*, presumably emitting modulators (cytokines and chemokines) that provoke ongoing focal inflammatory reactions. The result in some birds appears to be granuloma and giant cell formation at sites where engulfed particulates cannot be cleared from the secondary and tertiary bronchi.

- The pattem of response to embedded particulates does not include fibrosis in birds; mild focal fibrosis would have little fimctional impact on the non-inflating avian lung, but fibrosis might modestly increase respiratory effort if the air sacs are affected.
- Particles deposited in air sacs are likely to be engulfed by macrophages and cleared from the air sacs. The fate of the responding macrophages, and thus sites to which they might redistribute the LA particles, is not known.

3. There is no evidence that the lungs of wild avian species are anatomically, physiologically or immunologically more susceptible to inhaled particulates than mammalian lungs.

- Published assertions that "avian" lungs are more susceptible to particulate or pathogen damage than mammalian lungs consistently cite examples of the susceptibility of poultry (particularly broiler chickens and modem hybrid turkeys) to respiratory pathogens or to extremely challenging air quality when commercial growout facilities are poorly managed. Indeed, chickens bred for extremely rapid growth and meat production (broiler chickens) provide an excellent model of genetically-imposed cardio-pulmonary and immunological inadequacies. Broiler chicks typically hatch at a weight of 40 g and grow to 4 kg within 8 weeks. Thus in two months a broiler's body weight doubles and redoubles almost 7 times. If human infants grew at the same rate, their body weight would increase from 3 kg (6.6 lb) at birth to 310 kg (690 lb) by 2 months of age. The consequences are obvious: extremely rapid early growth in broilers imposes proportional challenges to their developmentally immature puhnonary, cardiovascular and immunological systems. Rapid grovvth triggers a suite of "metabolic diseases" attributable primarily to "outgrowing cardio-pulmonary capacities" or "impaired immuno-competency". Wild birds and the progenitors of modem poultry breeds are uniformly found to be considerably more robust tiian modem broiler chickens and hybrid turkeys (Wideman, 2000, 2001; Nganpiep and Maina, 2002; Wideman et al. 2004, 2007).
- Particulate deposition due to gravitational sedimentation and Brovmian diffiision most likely will occur where air velocities are low, particle residence time is prolonged, and at sites of air flow reversal. Accordingly, particles are highly likely to be deposited throughout the alveoli of mammalian lungs, precisely at the level where gas exchange must occur, and where membrane fibrosis is highly detrimental due to the loss of elasticity (alveoli must inflate and deflate during the respiratory cycle; fibrosis significantly increases respiratory effort in birds). In contrast, convective air flow does not penetrate the gas exchange capillaries of avian lungs, thus particle deposition within the air capillaries should be minimal or non-existent. Within the avian lung parenchyma, air flow is bidirectional in neopulmonic parabronchi which comprise 25%, at most, of the lung volume.
- Interstitial inflammation, granuloma development and giant cell formation are normal pattems of avian responses to puhnonary entrapment of particulates delivered either via the inspired air or via the bloodstream. Absent respiratory disease attributable to

pathogens, all available evidence indicates these intrapulmonary inflammatory responses have minimal impact on the function or viability of affected birds.

- Assuming equal levels of "exposure", the above considerations indicate that otherwise healthy mammals are likely to be *m*ore sensitive to particle inhalation than clinically healthy birds.

4. <u>Conclusion</u>: The experiments conducted by Nakaue, Pierson and Heifer (1982) and Bland, Nakue, Goeger and Heifer (1985) are highly instructive: 20 consecutive days of intensive aerosol exposure to volcanic ash particles of a respirable size did elicit intrapuhnonary histological changes but failed to alter any routine indices of broiler performance, nor was mortality affected. Broiler chickens are considerably less robust than wild birds (*vide supra*). Peacock and Peacock (1965) demonstrated that most adult Leghorn chickens survived several years after milligram quantities of asbestos fibers were instilled directly into their air sacs and (presumably) into the lung parenchyma. It is my opinion that some birds in the affected area are likely to exhibit histological evidence of intrapulmonary LA particulate exposure, but that little or no impact on the physiological function or viability of resident avian populations will be discemable.

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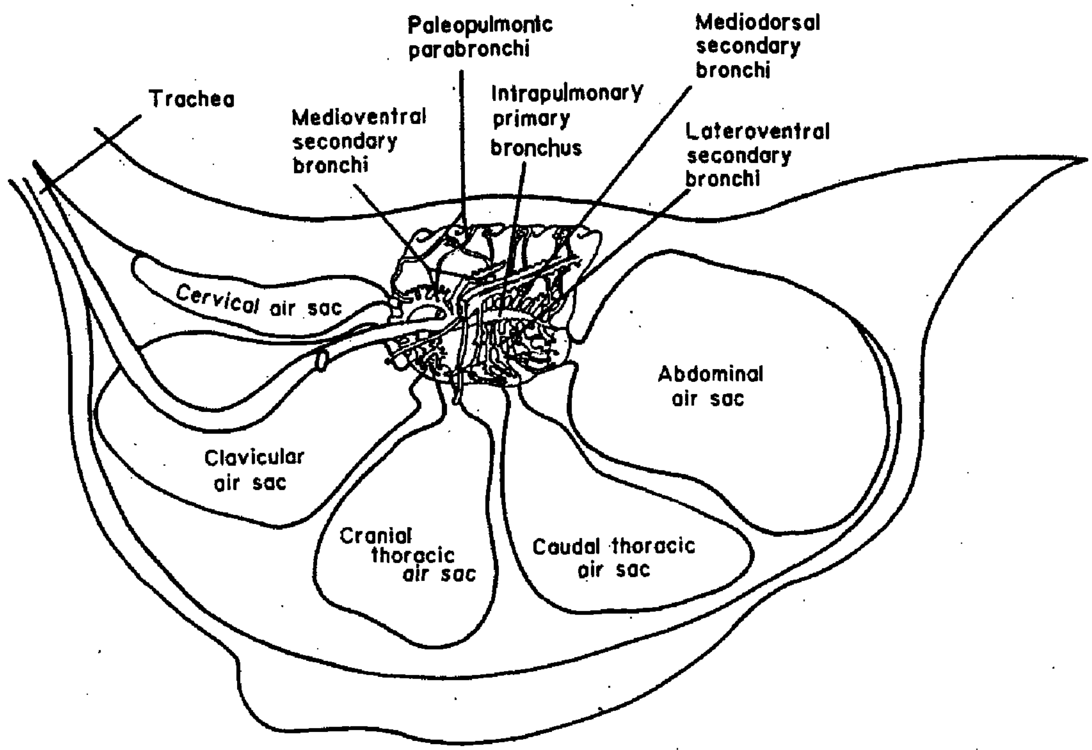
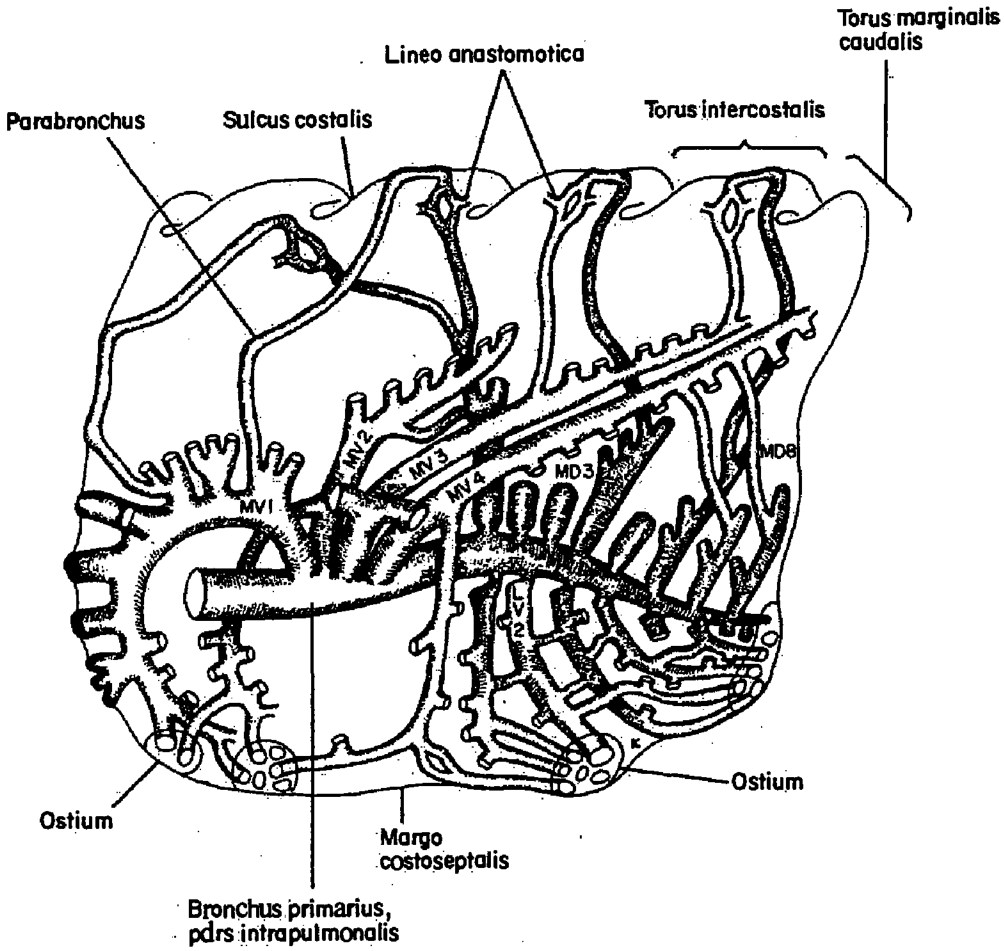


Figure 1. Schematic arrangement of avian lungs and air sacs. Deep within the thoracic cavity the trachea bifurcates at the syrinx (the avian organ of phonation) to form the right and left extrapulmonary primary bronchi. These bronchi penetrate the respective lungs to become the intrapulmonary primary bronchi. Within the lungs of domestic fowl, the medioventral, mediodorsal, lateroventral, and laterodorsal secondary bronchi branch from the intrapulmonary primary bronchus. The bronchi and air sacs connect via ostea.



Medial view of the right lung illustrating: the intrapulmonary primary bronchus; the medioventral (MV), mediodorsal (MD) and lateroventral (LV) secondary bronchi, paleopulmonic parabronchi (tertiary bronchi) connecting the MV and MD secondary bronchi; and, ostea (openings) to air sacs. The Costal sulcus represents a rib indentation.

Figure 2. Details of the primary and secondary bronchi within avian lungs. The intrapulmonary primary bronchus penetrates from the cranial to the caudal margins of the lung, opening caudally into the osteum of the abdominal air sac. Within the lungs the secondary bronchi branch from the intrapulmonary primary bronchus.

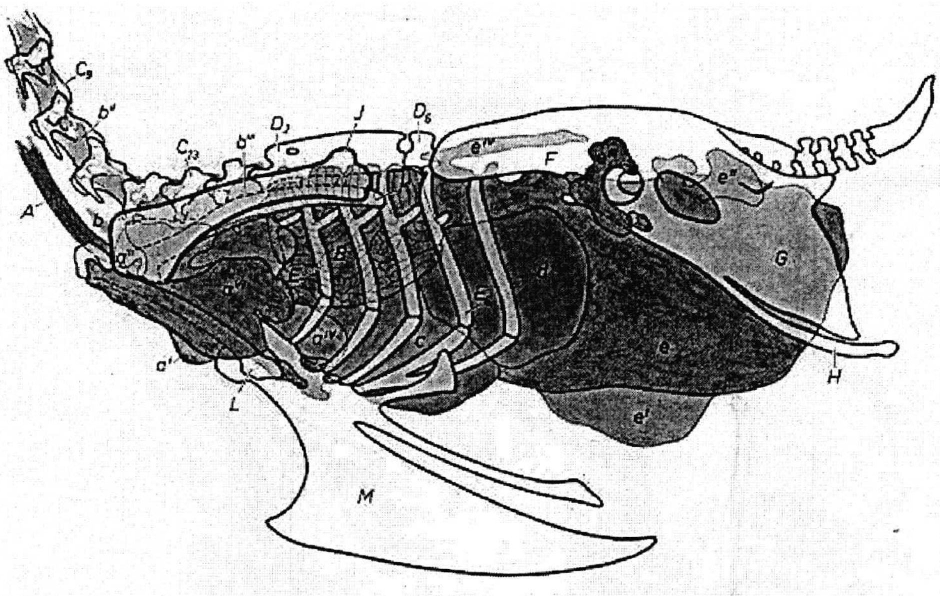


Fig. 68

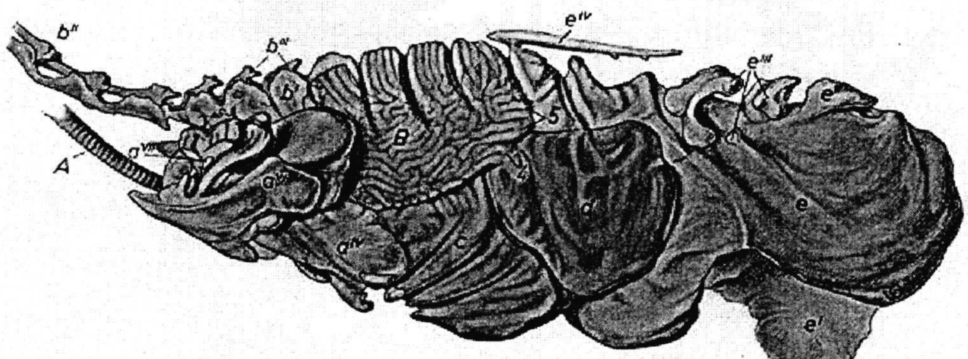


Fig. 69

Air sac system of the fowl (Weik, 1963).

A: trachea, B: lung; C: cervical vertebrae; D: thoracic vertebrae; E: ribs; F: ilium; G: ischium, H: pubis; J: humerus; K: scapula; L: coracoid; M: sternum; a: clavicular air sac; b: cervical air sacs; c: cranial thoracic air sac; d: caudal thoracic air sac; e: abdominal air sac

Figure 3. The non-inflating avian lungs (B) are partially encased by 5 ribs (E) as indicated by the costal sulci (indentations) in the dorsal-lateral aspect of the lungs. The air sacs are shown in their anatomically correct positions.

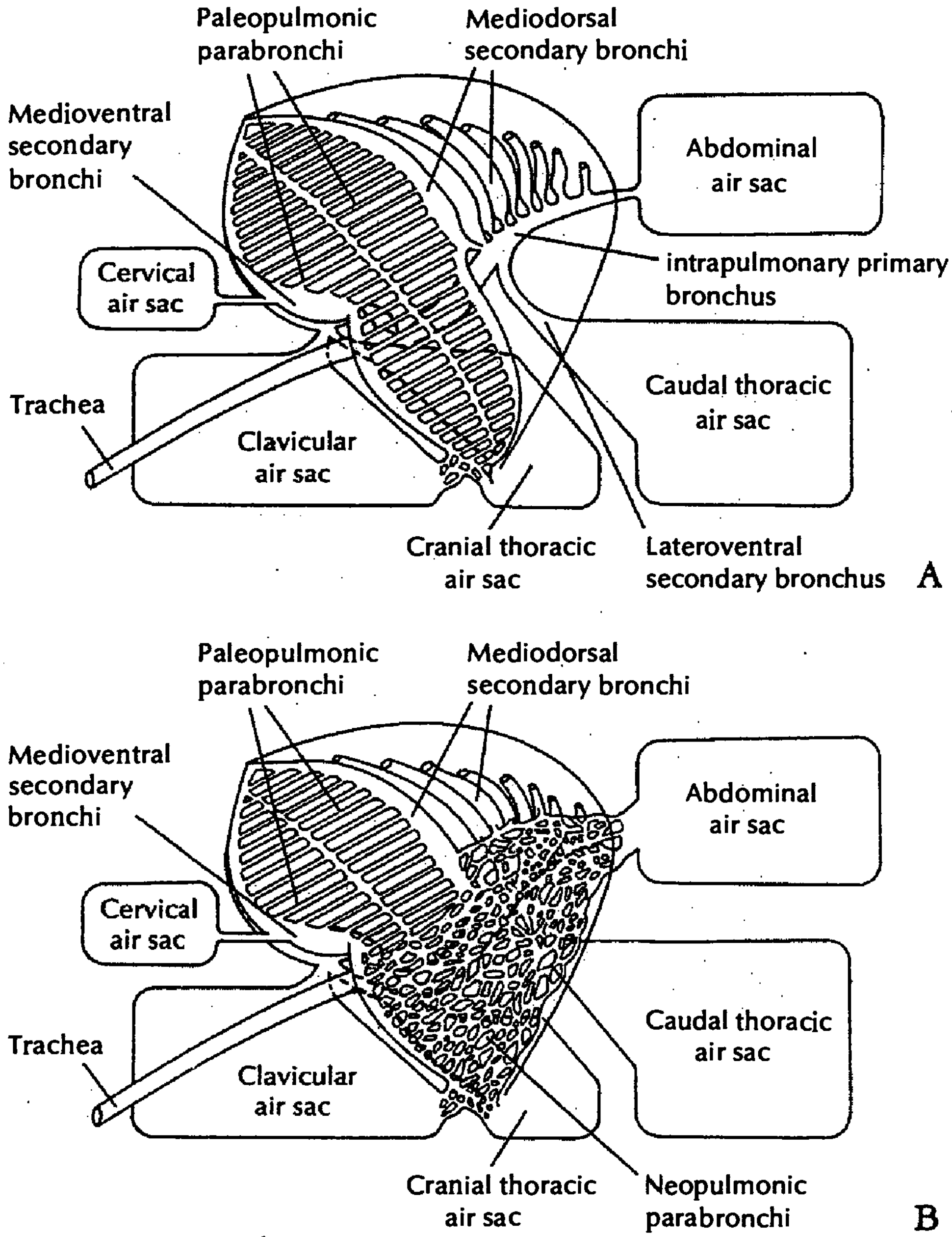


Figure 4. Scheme of the organization of the parabronchi in birds.
(A) Only paleopuhnonic parabronchi are present in some birds (e.g., penguin and emu). (B) In addition to paleopuhnonic parabronchi, a variably developed net of neopulmonic parabronchi is present in most birds (Duncker, 1972).

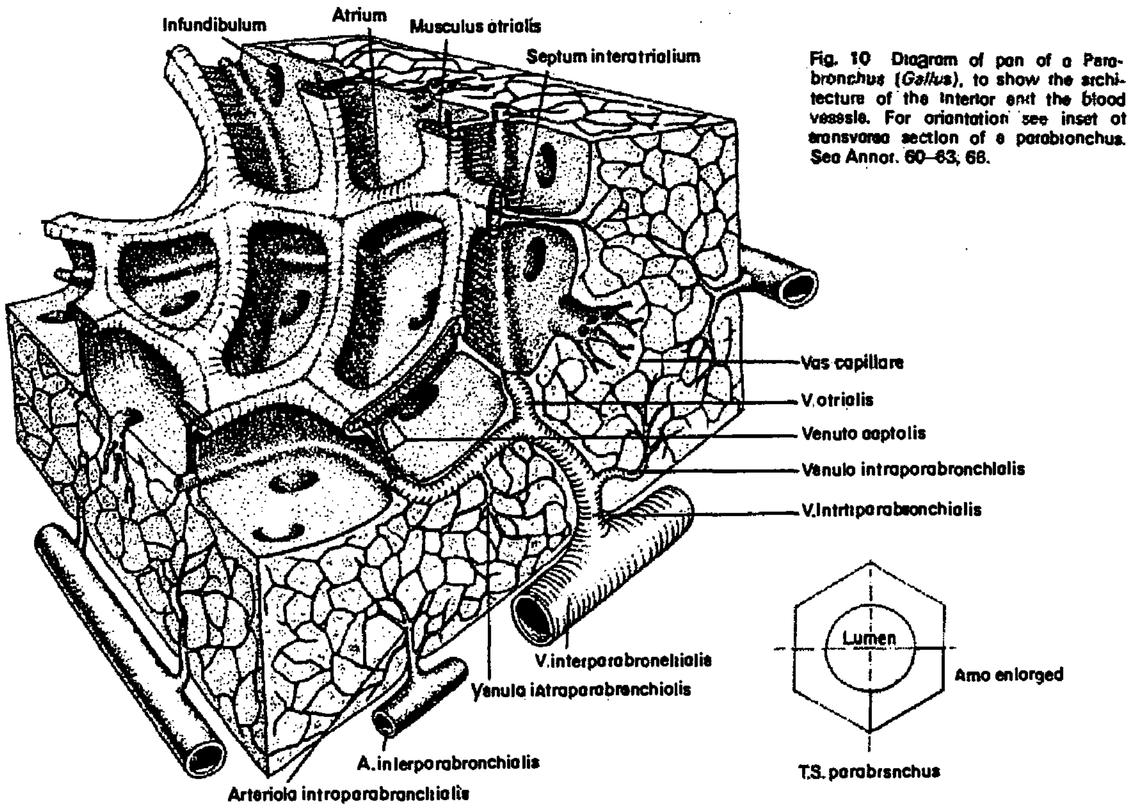


Figure 5. Section through part of the wall of a parabronchus. Atria 100-200μm in diameter form pockets projecting 50μm into the luminal wall. Spiral bands of smooth muscle (Musculus atrialis) underlie the parabronchial luminal epithelium and encircle the openiug to each atrium. One or more fhnnel-shaped infundibula penetrate from the atrial floor into the parabronchial wall, with muhiple freely anastomosing air capillaries originating from each infundibulum and radiating outward toward the periphery (outer boundary) of the parabronchus.

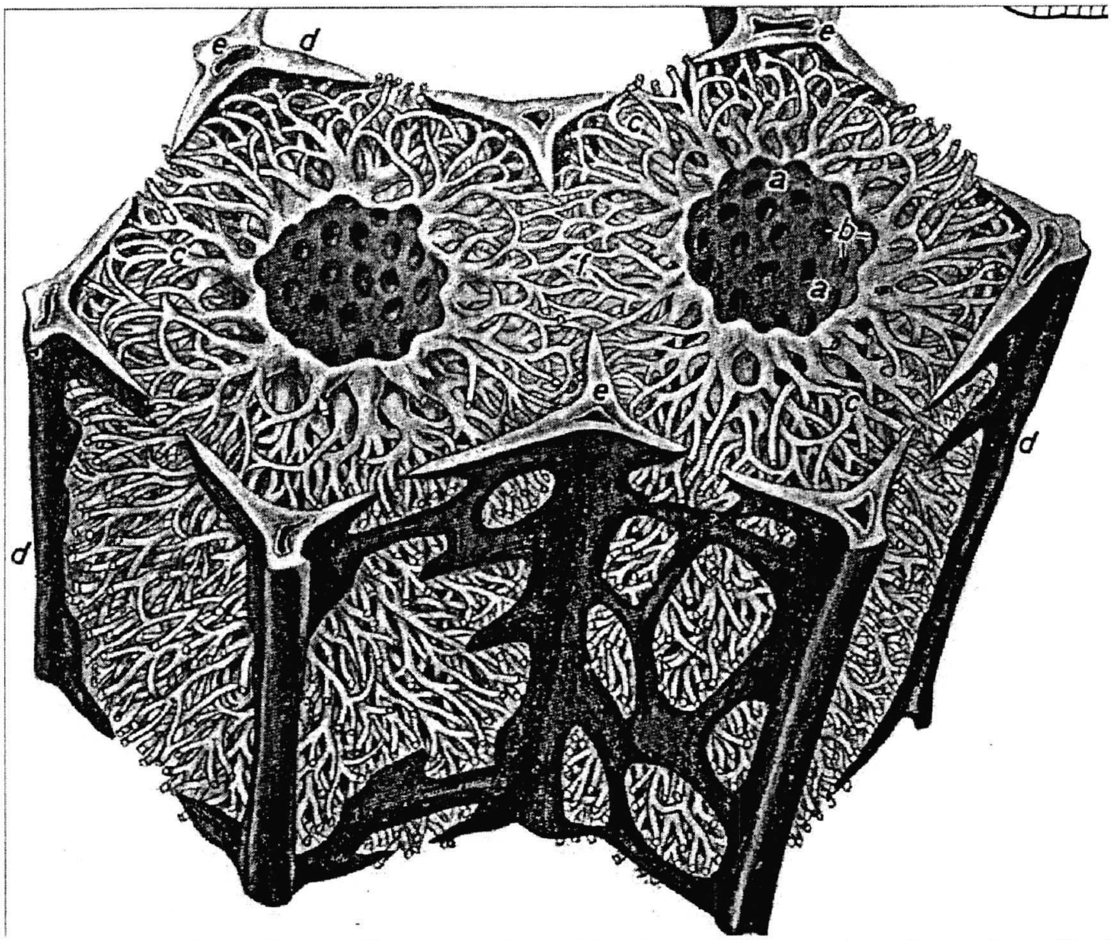


Figure 6. Section through two adjacent parabronchi. a: interatrial septa; b: atria; c: air capillaries; d: outer connective tissue septa; e: blood vessels; f: anastomotic connections between air capillaries. The **air capillaries** radiate outward toward the periphery (outer boundary) of the parabronchi.

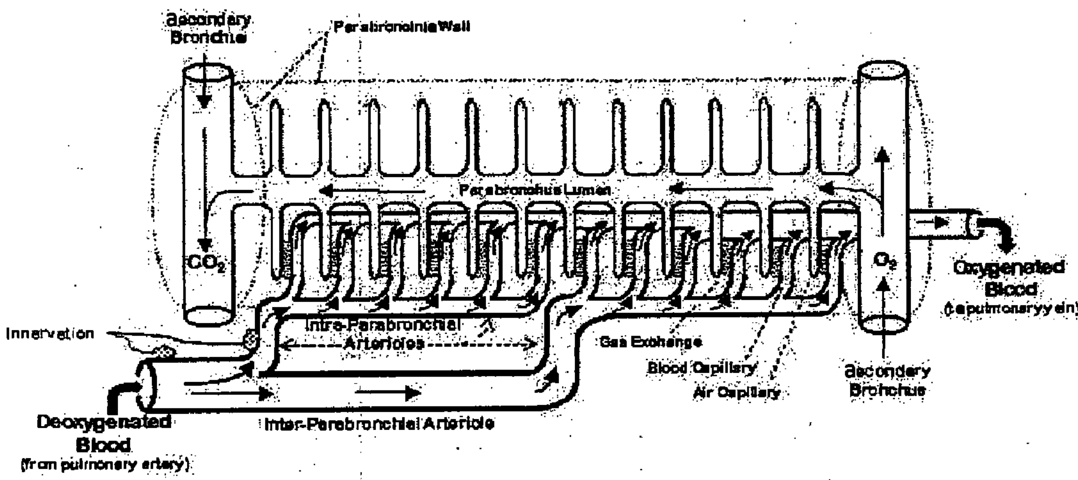


Figure 7. Interparabronchial arteries supply deoxygenated blood to Intraparabronchial arterioles branching inward into the parabronchial wall to form a net of blood capillaries surrounding each air capillary. Gas exchange occurs at the blood-gas barrier at the interface between blood capillaries and air capillaries. Venules collect the oxygenated blood at the base of the atria and infimdibula adjacent to the parabronchial lumen.

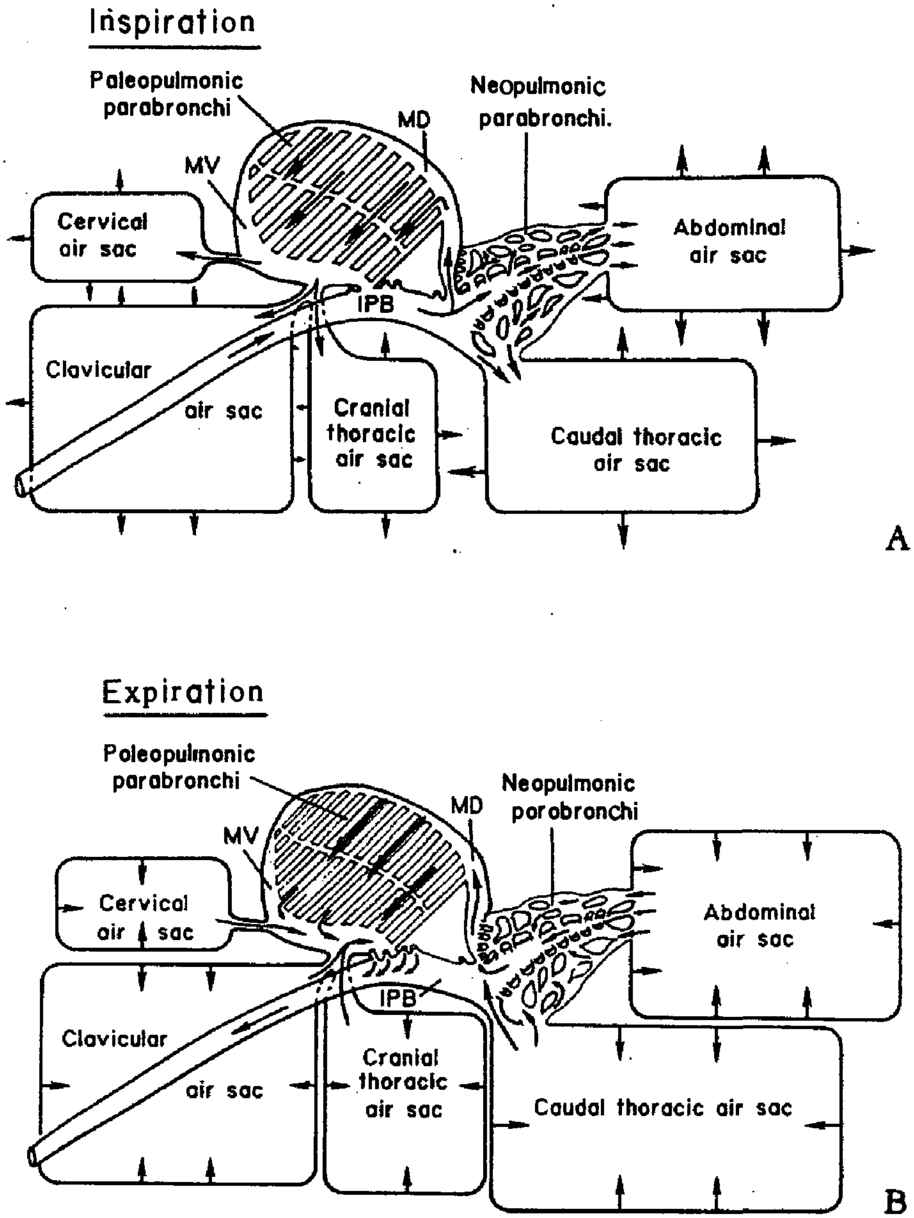


Figure 8. Schematic representation of the pathway of gas flow through the paleopulmonic and neopulmonic tertiary parabronchi during inspiration (A, upper panel) and expiration (B, lower panel). IPB: intrapulmonary prinary bronchus; MD: mediodorsal secondary bronchi; MV: medioventral secondary bronchi. Outward arrows on air sacs (upper panel) = inflation caused by negative thoraco-abdominal pressures (suction); Inward arrows on air sacs (lower panel) = deflation caused by positive thoraco-abdominal pressures. Arrows in primary, secondary and tertiary parabronchi show directions of convective air flow.

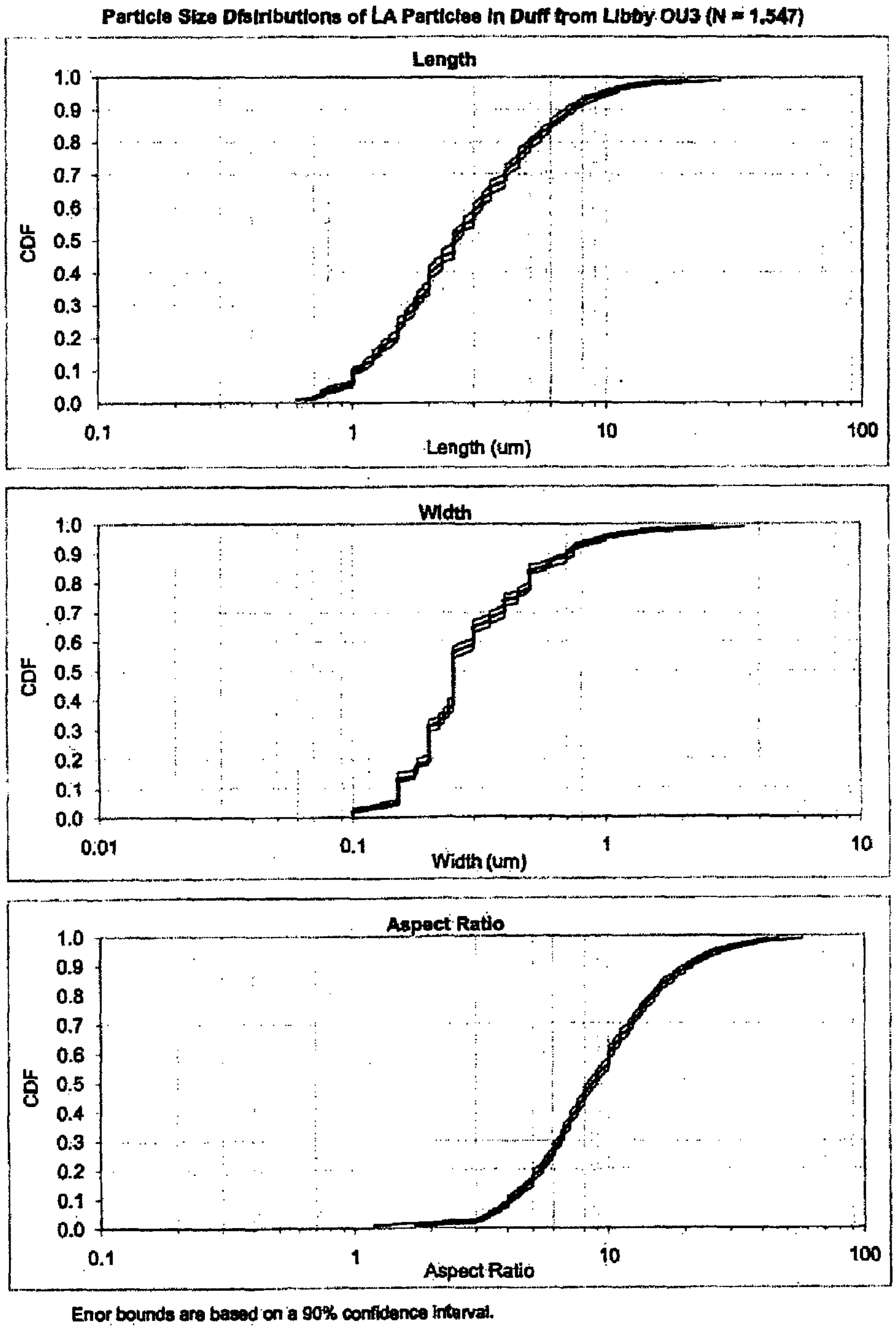


Figure 9. Particle size distributions for Libby Amphibole (LA) in duff.

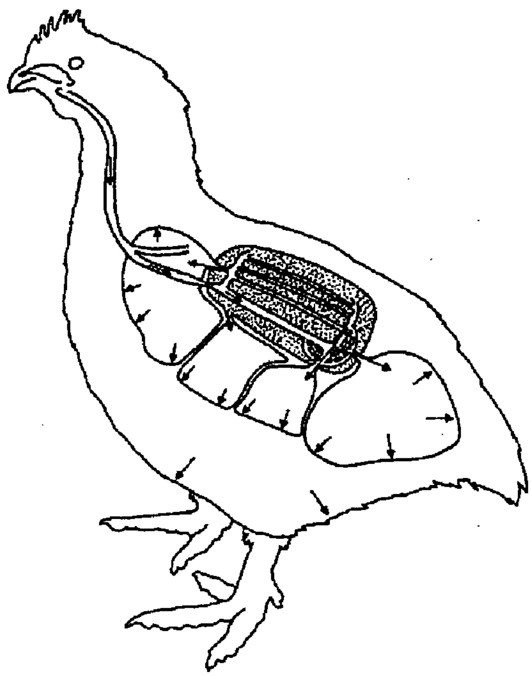


FIGURE 2. Palhway of gas flow in the avtan respiratory system during inspiration. Enlargement of the body cavity by inspiratory muscle action lowers pressure in the air sacs relative to that in the atmosphere and gas flows into the system. Gas does not enter the medioventral secondary bronchi, but passes into the mediodorsal secondary bronchi. Some of the gas passes through the paleopulmonic parabronchi, and the remainder passes into the neopulmonic parabronchi and caudal air sacs.

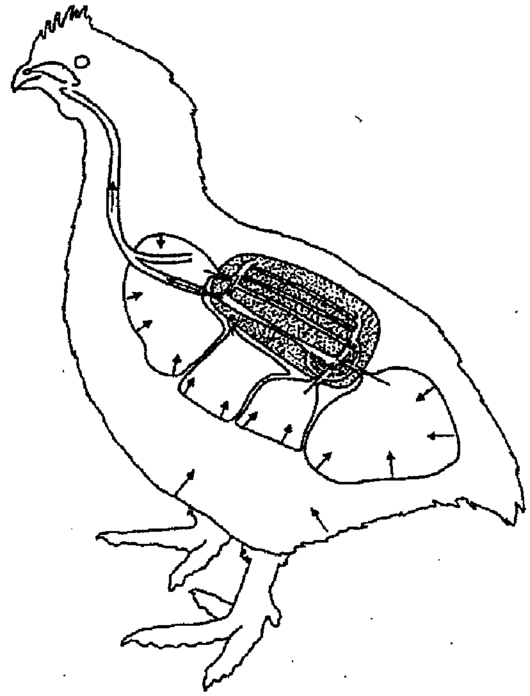


FIGURE 3. Pathway of gas flow in the avian respiratory system during expiration. Reduction in volume of the body cavity by expiratory muscle action increases pressure in the air sacs relative to that in the atmosphere and gas flows out of the system. Compression of intrapulmonary primary bronchus causes gas coming from the caudal air sacs to pass through neopulmonic parabronchi, into mediodorsal secondary bronchi and through the paleopulmonic parabronchi. Gas from the cranial air sacs does not pass through parabronchi on the way to the primary bronchus and trachea.

Figures from Fedde, 1998.

U.S. Environmental Protection Agency
Region 8
Denver, CO

AVIAN EXPERT CONSULTATION

Topic: Potential Avian Responses to Inhalation of Asbestos Fibers

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Insectivorous species that feed primarily on soil invertebrates.	American robin (*Turdus migratorius*) Northern Flicker (*Colaptes auratus*)
Avian species that feed primarily in trees on invertebrates.	American Three-toed Woodpecker (*Picoides dorsalis*) Black-backed Woodpecker (*Picoides arcticus*) Boreal Chickadee (*Poecile hudsonica*) Brown Creeper (*Certhia Americana*) Chestnut-backed Chickadee (*Poecile rufescens*) Golden-crowned Kinglet (*Regulus satrapa*) Lewis's Woodpecker (*Melanerpes lewis*)
Avian species that feed primarily on plant material and forage on the ground.	Brewer's Blackbird (*Euphagus cyanocephalus*) Chipping Sparrow (*Spizella passerina*) Horned Lark (*Eremophila alpestris*) Mourning Dove (*Zenaida macroura*) Ruffed Grouse (*Bonasa umbellus*)
Avian species that feed on other birds and small mammals.	American Kestrel (*Falco sparverius*) Barred Owl (*Strix varia*) Boreal Owl (*Aegolius funereus*) Flammulated Owl (*Otus flammeolus*) Great Gray Owl (*Strix nebulosa*) Great Horned Owl (*Bubo virginianus*) Northern Goshawk (*Accipiter gentilis*) Peregrine Falcon (*Falco peregrinus*)
Avian species that forage in along streams and ponds probing into sediments.	Marsh Wren (*Cistothorus palustris*) Spotted Sandpiper (*Actitis macularius*)
Avian species that feed on aquatic vegetation and sometimes aquatic invertebrates	American Coot (*Fulica americana*) Blue-winged Teal (*Anas discors*) Green-winged Teal (*Anas crecca*) Harlequin Duck (*Histrionicus histrionicus*) Mallard (*Anas platyrhynchos*)
Piscivorous species that feed primarily on fish and some invertebrates.	Bald Eagle (*Haliaeetus leucocephalus*) Belted kingfisher (*Ceryle alcyon*) Common Loon (*Gavia immer*) Great Blue Heron (Ardea herodias)

Sensitivity of Birds to Libby Amphibole

As part of the 2009 Phase III sampling program for the Libby Mine Site, bird sampling was considered to determine if birds are potentially at risk irom Libby amphibole fibers. There are no studies of the effects of asbestos on birds, and only one published study on particle deposition in the avian respiratory tract. Therefore, empirical comparisons of sensitivity of birds to manunals following exposure to Libby amphibole are not possible. However, because of differences between the physiology of the avian and mammallan respiratory systems, gastrointestinal tracts, and kidneys, it is probable that birds will be less affected than small mammals by inhalation of the Libby amphibole. The comparisons are described below.

Respiratory Comparisons

Asbestos fibers are known to lodge in the lungs of mammals, with the long, thin Libby amphibole fibers depositing mainly in the lower airways and alveolar regions (ATSDR, 2001). As a foreign antigen, tiney attract alveolar macrophages and pulmonary neutrophils, and interact with epithelial cells and pleural mesothellal cells, setting off an inflammatory cascade response and eventually a walling-off of the fiber irom the Iumg tissue. This results in pulmonary interstitial fibrosis and collegen deposition, with progressive lung stiffening and thickening and calcification of the pleura and, eventually, a reduced abillty of the lungs to expand, thus decreasing gas exchange and oxygenation of the blood. (ATSDR, 2001). Production of reactive oxygen and/or nitrogen species may result in carcinogenesis, particularly of the pleural mesothellum.

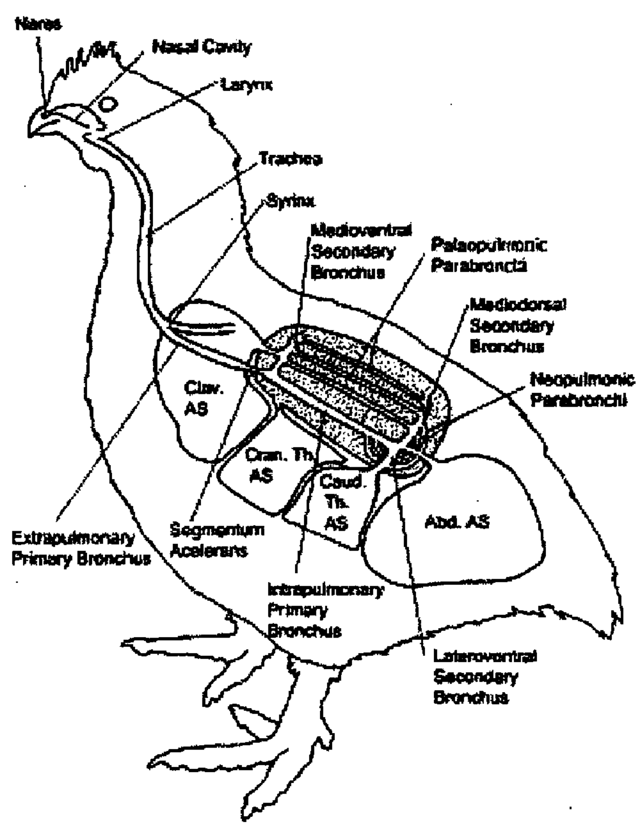

FIGURE 1. General organization of the respiratory system in the chicken. Clav. AS - clavicular air sac; cran. th. AS - cranial thoracic air sac; caud. th. AS - caudal thoracic air sac; Abd. AS - abdominal air sac.

Birds, on the other hand, have relatively small lungs that do not expand upon inhalation (Brovm et al., 1997). Instead, the air is pulled through the lungs by the bellows action of the air sacs (see Figure 1; irom Fedde 1998). The air flows through the lungs in a single direction on both the inhalation and exhalation parts of the breathing cycle. There are no blind alveolar sacs, as in mammalian lungs, and the air simply passes through a series of smaller and smaller bronchi, which are highly vascularized for efficient gas exchange. Because the lungs do not expand during inhalation, pleural thickening and calcification (if any) or interstitial fibrosis that may be caused by asbestos fibers would have no effect on respiratory efficiency.

Although birds have prominent bronchus-associated Iymphoid tissue, they lack surface alveolar macrophages (Reese et al., 2006). Instead, the phagocytic function of macrophages is fulfilled by epithelial cells. Particles move into liposomes wifhin the epithelial cells or they may move through to the interstitium, where they are picked up by interstitial macrophages (Reese et al., 2006). The lack of alveolar macrophages suggests that birds may not respond as aggressively to particles that remain within the lmgs, and therefore may have less interstitial fibrosis. Further, mid- to large sized particles ($\geq$ 10 ȷun) deposit primarily in the abdominal air sacs and caudal (rear) bronchi (Steams et al., 1987) rather than in the lung parenchyma. Because the air sacs are made of connective tissue with very little vascularization, inflammation and fibrosis as a result of fiber deposition does not appear likely.

Birds have a high requirement for oxygen, as flight is fhe most metabolically expensive form of locomotion on a unit-time basis (Brown et al., 1997). However, the effective ventilation in birds under resting conditions is 30 – 160% higher than mammals of comparable size, indicating the much higher gas exchange efficiency of the avian lung (Brovm et al., 1997).

Gastrointestinal and Kidney Comparisons

The avian gastrointestinal (GI) tract is simllar in structure to that in mammals, so likely wlll experience the same type of response to asbestos ingestion. However, birds do not have an epithellal mucocilliary transport mechanism for removing particles irom their trachea and upper bronchi (Fedde, 1998), and so may experience less GI exposure through pulmonary clearance than do mammals. Although the gross morphology of the avian kidney differs irom that of mammals, the nephron is still the functional unit, with the same basic structure of glomerull that filter the blood and renal tubules to reabsorb water. Thus, there is no reason to belleve that the sensitivity of response to renal asbestos exposures would differ between birds and mammals.

Summary

In summary, birds are less likely than smali mammals to suffer irom respiratory effects of Libby amphibole because:

- Their lungs do no expand during breathing so pleural thickening or calcification is not a problem;
- The flow-through construction of their lungs would result in particle deposition occurring primarily in the air sacs;
- Air sacs are not very vascularized, so inflammation generally does not occur; and
- They do not have alveolar macrophages, so may experience a reduced intestinal inflammatory response.

Birds are not likely to differ irom mammals in regard to sensitivity of gastrointestinal tract or kidney exposures.

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Reese, S., G. Dalamani, and B. Kaspers. 2006. The avian lung-associated immune system: a review. Veterinary Research. 37:311-324.

Steams, R.C., G.M. Bamas, M. Walski, and J.D. Barin. 1987. Deposition and phagocytosis of inhaled particles in the gas exchange region of the duck, Anals platyrhynchos. Respir. Physiol. 67:23-36.

U.S. Environmental Protection Agency
Region 8
Denver, CO

AVIAN EXPERT CONSULTATION

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LIBBY OU3 BTAG
December 8, 2012

Fish Population and Habitat Data Evaluation

Purpose

- Decide if current habitat data provide a convincing and sufficient explanation for observed lower fish population estimates in Lower Rainy Creek.

Overview

- Review fish population data
- Habitat data evaluation
- Conclusions
- Discussion

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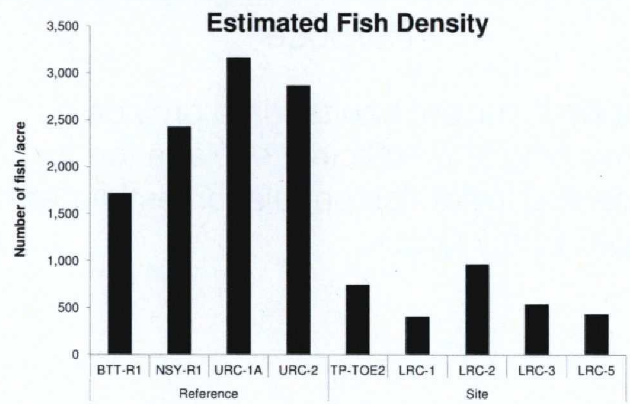

LA concentrations generally higher in LRC ⟶

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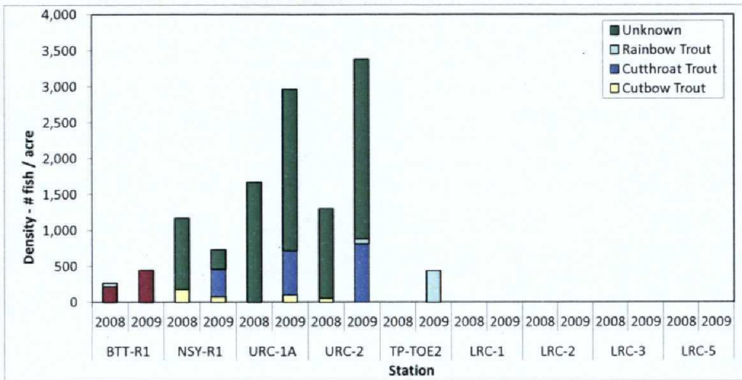
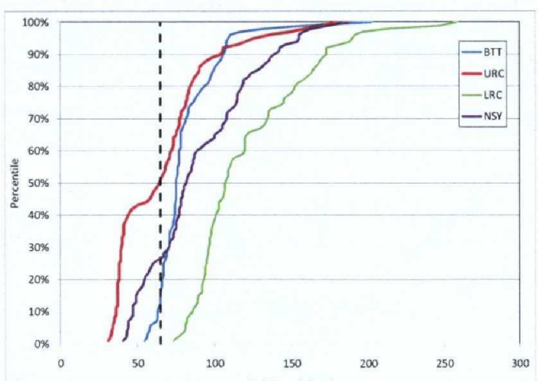
Young of the Year



LA concentrations generally higher in LRC →

CDF of October Fish Length Data (2008-2009 electroshocking data)



LRC size distribution is right shifted compared to reference

Key Fish Habitat Metrics and Optimal Ranges (identified July 2010 BTAG)

Habitat Metric	Rainbow		Cutthroat	
	Range	Source	Range	Source
Spawning Gravel Size (mm)	15 - 100	2	15- 80	5
% Fines (< 2 mm)	<5 - 20	2	<5 - 15	3
Temperature (^{0}C)	7 - 18	2	7 - 16	3
Pool Depth (cm)	30-100	1,2	30 - 100	1,2
Large Woody Debris (%)	≥ 25	2	14 to ≥ 22	3
DO (mg/L)	7 - 9	4	7 - 9	3
Velocity for Embryo Dev (cm/sec)	30 - 70	4	30 - 60	3
% Pools (late growing season)	35 - 65	4	35 - 65	3

1 - Harig and Fausch 2002

2 - Adams et al 2008

3 - Department of Interior FWS Cutthroat 1982

4 - Department of Interior FWS Rainbow 1984

5 - Varley and Gresswell 1988

HSI Overview

Embryo
- Avg max temp
- Avg min DO
- Avg water velocity
- Avg gravel size in spawning areas
- % Riffle fines

Fry
- % Substrate size class
- % Pools
- % Riffle fines

Juvenile
- % In-stream cover
- % Pools
- Pool class rating

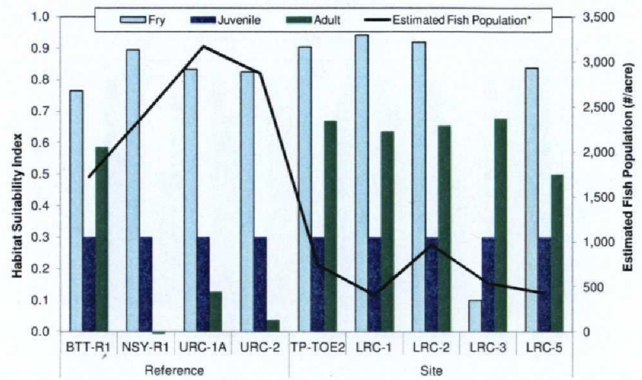
Adult
- Avg Thalweg water depth
- % In-stream cover
- % Pools
- Pool class rating

Other
- Avg max temp
- Avg min DO
- pH
- Avg base flow
- Dominant substrate type
- % Streamside veg. index
- % Stable streamside veg.
- % Fines in riffle run areas
- % Midday shade

Lifestage HSI

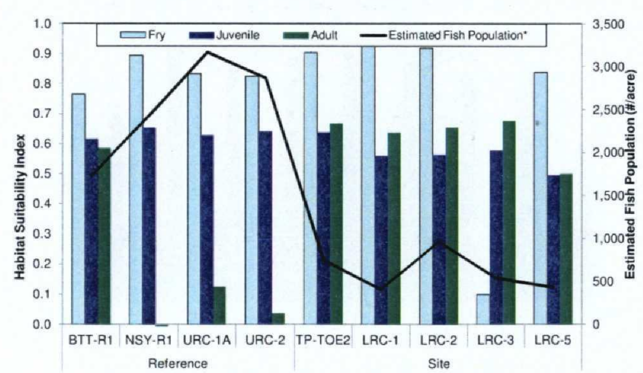
Species HSI

Habitat Suitability Index for the Fry, Juvenile and Adult Life Stages



*Estimate is based on the average 2008 and 2009 MLE population estimate for fish greater than 65 mm.

Modified Habitat Suitability Index for the Fry, Juvenile and Adult Life Stages



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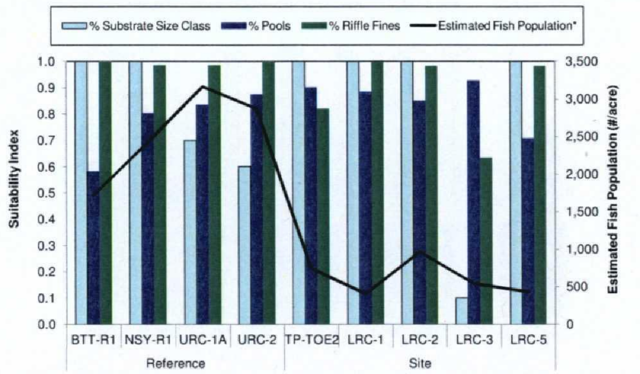
Habitat Suitability Index Variables for the Fry Life Stage



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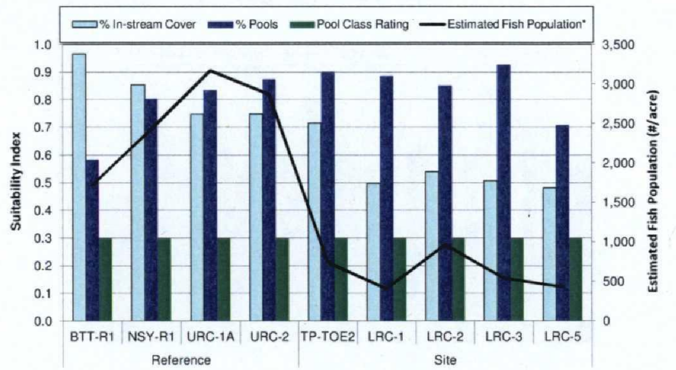
Habitat Suitability Index Variables for the Juvenile Life Stage



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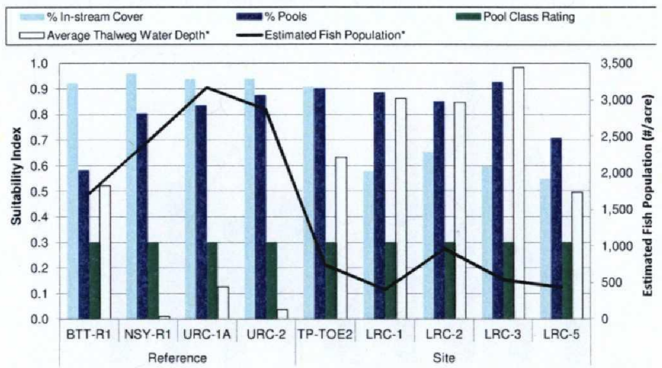
Habitat Suitability Index Variables for the Adult Life Stage



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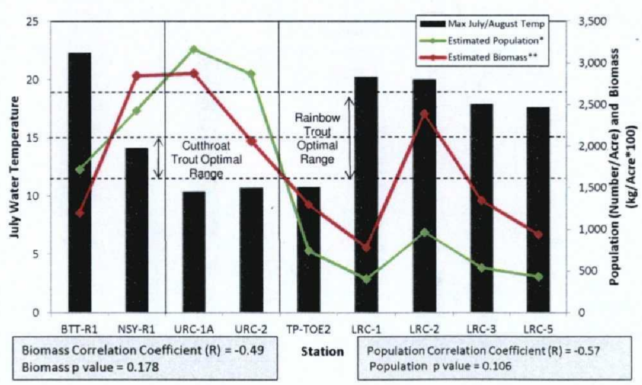
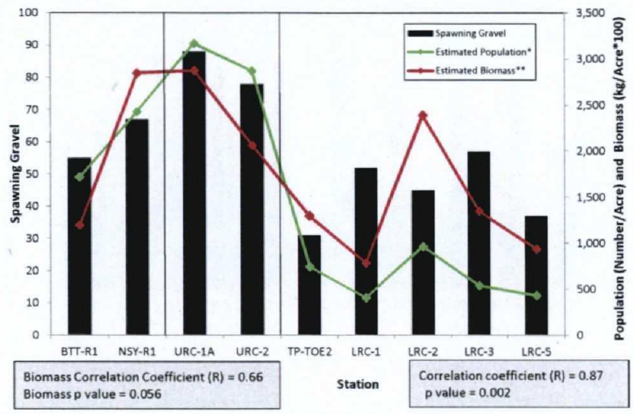
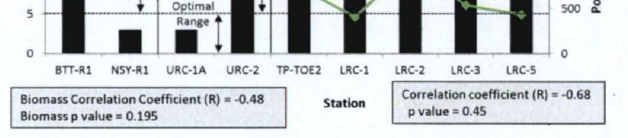
Maximum July/August Water Temperature



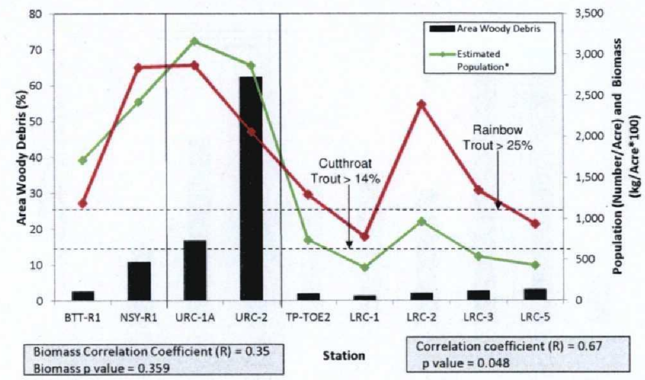
Percent Spawning Gravel (2-64 mm)



Percent Fines(< 2mm)

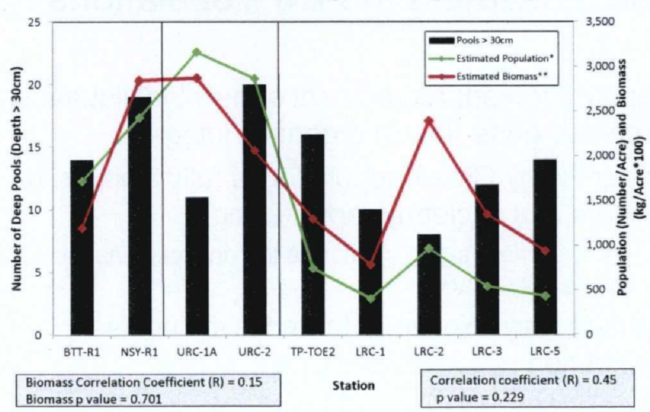


Percent Woody Debris

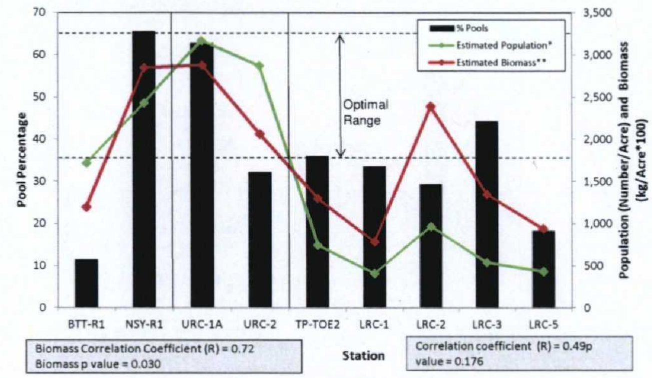


Optimal of ≥ 25% for rainbow trout and 14% to ≥ 22% for cutthroat trout.

Number of Deep Pools (>30cm)



Percent Pools



Optimal % pools for rainbow and cutthroat trout in late growing season (35% - 65%).

Role of Barriers in Fish Populations

- Barriers prevent recruitment of new individuals and decrease gene flow and genetic integrity
- Upper Rainy Creek population is fully isolated by barriers, but is clearly reproducing.
  - Thus, barriers alone cannot be the only explanation for lower fish density in LRC
- Barriers may explain differences in species distribution

Conclusions

- Lifestage HSI does not reveal significant differences between reaches.
- Some individual habitat factors are likely contributing to the decreased population in LRC other are not.
- Additional habitat data will unlikely explain population differences between reaches.

Does habitat characterization explain observed population differences?